DEMOGRAPHIC AND ENVIRONMENTAL DETERMINANTS OF REPRODUCTIVE SUCCESS IN *SYNTHYRIS BULLII,* A RARE ENDEMIC SPECIES

BY

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DISSERTATION

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ABSTRACT

In recent decades, the conversion of natural habitat to human use has proceeded at a greatly accelerated pace. This habitat destruction and degradation has resulted in the loss of much of the world's biodiversity. Some populations have been preserved in remaining habitat reserves, but their persistence may be further threatened by degradation and interruption of historic ecological processes. In the case of plants, the loss of pollinator mutualists and changing abiotic conditions may comprise the ability of plants to reproduce and maintain viable populations. My thesis research examines several aspects of the demographic and environmental factors that may influence the reproductive ecology of *Synthyris bullii*, a rare perennial species endemic to North American prairies and savannas. In Chapter 1, I explore the relationship between reproductive success and floral quantity versus quality. The results of this study show that inflorescence size correlates positively with population size, and that both factors play important roles in determining reproductive output. Chapter 2 examines the response of reproductive morphology to different habitat conditions, and shows that there are tradeoffs in inflorescence and flower characteristics associated with each habitat type. I continue to explore the importance of habitat type in Chapter 3, using an experimental manipulation to determine pollinator visitation at sites that vary in woody encroachment. The results show that reproduction is positively associated with more open habitats, and that pollen quality may partly explain the reproductive success in these populations. Taken together, the results of these studies show that habitat degradation in North American prairies is associated with changes in flower morphology, reduced pollinator visitation, and loss of reproductive output for *S. bullii*.

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CHAPTER 1

GENERAL INTRODUCTION

The previous decades of human history have been characterized by the unprecedented destruction of natural habitat across the world, leading to a widespread loss of biodiversity (Wilson 1989, Wilcove et al. 1998, Pimm and Raven 2000, Hoekstra et al. 2005). Aside from intensive conversion of the landscape for urbanization, agriculture, and resource extraction, modern human activities also interfere with historic ecological processes and delicately coevolved species interactions (Hobbs and Harris 2001, Fischer and Lindenmayer 2007, Dunn et al. 2009). In North America, the cessation of periodic fires and grazing disturbance in temperate grasslands, such as prairie and savanna, have contributed to habitat degradation and the subsequent loss of native flora and fauna (Leach and Givnish 1996, Briggs et al. 2002, Ratajczak et al. 2012).

While habitat destruction and degradation drive patterns of extinction across the world (Foley et al. 2005, Mix et al. 2006, Krauss et al. 2010), the ability of populations to persist in remaining habitat patches depends on successful reproduction and recruitment. In conservation biology, there is particular emphasis to understand the mechanisms that determine population growth as populations that remain in low abundance over successive generations are at increased risk of extirpation from random stochastic events (Lande 1993, Matthies et al. 2004). Previous research has also found that small populations are especially susceptible to inbreeding and its associated fitness consequences, which may further compromise the viability of these populations (Oostermeijer et al. 1994, Young et al. 1996, Newman and Pilson 1997, Reed 2005). Thus, a major aim of conservation is to grow and maintain large populations (Shaffer 1981, Lande 1988, Ellstrand & Elam 1993, Lienert 2004), which means improving reproductive output.

From a practical standpoint, management plans for endangered species must also incorporate an understanding of the target species' reproductive biology, otherwise recovery efforts may be made in vain.

However, this task is fraught with challenges associated with the specific biology and ecology of organisms. Unlike animals, plants are sessile by nature and must overcome special challenges in the face of habitat destruction and fragmentation (Lienert 2004, Kremen et al 2007). Specifically, many plants rely on animal pollinators to facilitate outcrossing by dispersing pollen to other individuals (Wilcock and Neiland 2002, Young et al. 1996, Kolb 2008, Potts et al. 2010). The loss of these mutualists can lead to severe negative consequences for reproductive processes in populations (Kolb 2008, Dunn et al. 2009). As habitats degrade and abiotic conditions become inhospitable, plants may also become deprived of the resources necessary to support reproductive functions, such as production of flowers, fruit, and viable seeds (Harris and Scott 1969, Weijschedé et al. 2006).

Aside from the challenges associated with their sessile natures, plants also generally have more complex reproductive systems, and this may be especially the case for rare plants. From one species to the next, plants can show considerable variation in their breeding systems, ranging from obligate outcrossing to asexual reproduction via underground ramets (Berge et al. 1998, Wilcock and Neiland 2002). However, rare plants are thought to exhibit atypical reproductive traits, and these traits are thought to partially explain their naturally low abundances or inability to recover after catastrophic losses (DeMauro 1993, Oostermeijer et al. 2003). Because of these specific qualities, generalities derived from studies on common species may not appropriately apply to rare species, and studies should instead use rare species when examining reproductive ecology and responses to habitat fragmentation and degradation.

For my thesis research, I used a combination of field surveys, experiments, and statistical modeling to explore traditional determinants of reproductive success within a conservation context. A specific focus of this research is to determine the demographic and environmental conditions that are associated with high versus low reproductive output in rare populations. The conservation implications of these results are important for conservation biologists and land managers who wish to better understand how reproduction in rare species is influenced by degradation of native habitat.

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CHAPTER 2

CAN FLORAL DISPLAY SIZE COMPENSATE FOR ALLEE EFFECTS CAUSED BY LOW POPULATION ABUNDANCE AND DENSITY IN *SYNTHYRIS BULLII* (PLANTAGINACEAE), A RARE SPECIES?

ABSTRACT

Premise of the Study. Conservation seeks to address the issues of small population size, and the reproductive limitations confronting these populations. Sparse, small plant populations often suffer Allee effects such as pollinator limitation. However, some studies show that plants in sparse populations experience reduced resource competition. As a result, these plants may produce larger floral displays, which are also predicted to attract pollinators. The negative impacts from reduced floral quantity may thus be offset by improved floral quality.

Methods. In a 2 year field study, population abundance and density were quantified for 24 populations of *Synthyris bullii* (Plantaginaceae), a rare prairie endemic. In each population, data was collected on inflorescence size, fruit/seed set, and seed germination.

Key Results. Inflorescence size had a positive relationship with population inflorescence abundance and density. Fruit set and germination responded positively to floral quality (i.e., flower density). In comparison, seed set showed a positive relationship with only floral quantity (i.e., population abundance).

Conclusions. Contrary to my predictions, inflorescence size and population size were not inversely related. While attractive floral displays in sparse populations potentially compensate in terms of fruit set, population abundance nevertheless plays an important role in seed set. Because floral quality and quantity differ in their ability to explain reproductive outcomes,

studies should examine reproduction at several stages, otherwise the impacts of population size may be overlooked. Allee effects manifesting at a critical stage of reproduction, such as seed production, may act as a bottleneck impeding successful recruitment.

INTRODUCTION

Significant research in plant reproductive ecology attempts to characterize population traits that enhance plant attractiveness for pollinators and, consequently, reproductive success (Klinkhammer and de Jong 1990, Kunin 1997, Kirchner et al. 2005, Dauber et al. 2010). Traditionally, such studies examine abundance (i.e., population size) and spatial proximity among individuals (i.e., population density) because these factors play important roles in attracting pollinators (Kunin 1997). Widespread habitat destruction and fragmentation due to anthropogenic activities can compromise plant-pollinator relationships as a result of altered plant abundances and spatial patterns (Olesen and Jain 1994, Mustajärvi et al. 2001, Jacquemyn et al. 2002). Therefore, understanding the influence of population size and density on reproductive success is of particular importance, especially for endangered or rare species.

Because pollinators base foraging decisions on their ability to efficiently maximize food acquisition (Harder et al. 2001), pollinators are likely to choose plant populations where flowers are abundant and easy to access. From a pollinator's perspective, larger populations represent greater potential food resources (Waites and Ågren 2004). Further, increased population density facilitates pollinator movement by minimizing distance among plants, and thus reduces time and energy requirements while foraging (Gerber 1985, Stacy et al. 1996, Franceschinelli and Bawa 2000, Wilcock and Neiland 2002). As the likelihood of pollinator visits increases, plants in larger, denser populations are predicted to receive pollen more frequently (Jennersten 1988,

Waites and Ågren 2004). Pollinator preferences for larger, denser patches can therefore explain the positive relationship between plant reproductive success and population size and density (Kunin 1993, Molano-Flores and Hendrix 1999, Morgan 1999, Bosch and Waser 2001, Mustajärvi et al. 2001).

Pollinator foraging behavior can also be influenced by cues at more localized levels, such as individual floral traits, and population size and density are not necessarily the most reliable predictors of pollinator visitation (Kunin 1997, Bosch and Waser 2001, Grindeland et al. 2005). Although smaller, sparser populations are predicted to receive fewer visits, their attractiveness to pollinators may improve when plants produce large floral displays (Mustajärvi et al. 2001, Grindeland et al. 2005). This suggests that pollinators assess floral resources in terms of both quantity and quality.

While some plant species cope successfully with habitat changes associated with fragmentation, others are declining and becoming increasingly rare (Fischer and Matthies 1998, Matthies et al. 2004). The effect of population size and density on reproductive success is of particular interest in conservation because rare species, in many cases, suffer from low abundances (Rabinowitz 1981). As a result of their small populations, rare species are vulnerable to Allee effects, such as decreased pollinator visitation and decreased probability of receiving pollen from a suitable donor (Wilcock and Neiland 2002, Oostermeijer et al. 2003). Reproductive failure that results from reduced population size may impede recovery for these species, resulting in permanently small populations that are vulnerable to extinction by stochastic events (Jacquemyn et al. 2002, Matthies et al. 2004).

However, previous studies show that decreased plant density can lead to the production of larger floral displays in individuals (Mustajärvi et al. 2001, Grindeland et al. 2005), thus rare

plants could mitigate the negative reproductive impacts of low population abundance. The production of larger floral displays may not be a direct compensatory response of sparse populations to increase population attractiveness, per se, but the unintended outcome of decreased resource competition experienced by individuals in the population. Plants growing in high density populations are assumed to undergo more intense intraspecific competition for light and nutrients (Meekins and McCarthy 2000), and the costs and limitations imposed by resource competition may affect the quality and quantity of flowers produced by these plants (Mustajärvi et al. 2001). Therefore, in lower density populations, the decreased competition for resources is predicted to result in larger floral displays, which may consequently improve these plants' opportunity for reproduction.

This potential ability of plants to compensate for population-level deficiencies in floral quantity represents an important area for conservation research as it may enhance the ability of plants in small populations to attract pollinators and minimize Allee effects. Because natural populations often lack sufficient population and spatial variation to answer questions on abundance and density, many past studies use experimental methods (i.e., plants arranged in arrays) to manipulate plant density (Kunin 1993, Bosch and Waser 2001). However, such methods are potentially intrusive and damaging to sensitive rare species, and legal protections associated with endangered species often prevent experiments involving extensive manipulations. Thus, studies on these species must use many natural populations to adequately capture variations in population size and density. Moreover, natural populations reflect true distributions of rare species in their native environments, and can provide important insight into the ways that reproduction in actual populations respond to variations in population size and density.

Therefore, the objective of my study was to examine reproduction and fitness in several populations of an endangered species to determine if increased floral quality (e.g., inflorescence height, floral density of each inflorescence) can compensate for deficiencies in floral quantity (e.g., population abundance and density). First, I determined if there was a relationship between floral quality and quantity (i.e., if inflorescence size varied in relation to the size of a population). This step was necessary to confirm findings from past studies that plants in smaller, sparser populations produce larger floral displays. Next, I examined the relationship between reproduction and floral quality and quantity. For compensation to occur, I predicted that there would be a positive relationship between inflorescence size and reproductive success independent of population abundance/density. Alternatively, if plants were unable to compensate, then I predicted population abundance/density to have a much stronger effect on reproductive success independent of inflorescence size. Flowering, reproductive, and fitness data were collected for 24 natural populations of *Synthyris bullii*, a rare species endemic to the Midwestern United States. These data allowed me to explore the associations between floral quantity and quality that can explain reproductive differences among populations.

MATERIALS AND METHODS

Study Species—*Synthyris bullii* (Eaton) A. Heller (Plantaginaceae; Kittentail; synonym *Besseya bullii* (Eaton) Rydb. [*Scrophulariaceae*]), is a prairie-savanna perennial forb endemic to the Midwestern region of the United States of America (Gleason and Cronquist 1991). It is characterized by a large basal rosette and one or more spiked inflorescences (Gleason and Cronquist 1991). *Synthyris bullii* is self-compatible and pollinated primarily by bees (McKone et al. 1995, K. Chi pers. obs.). Number of flowering individuals in *S. bullii* populations can range

from zero to thousands (Appendices A and B). Inflorescences can have 10 to 130 flowers per spike, and one to nine inflorescences per plant (K. Chi pers. obs.). However, most flowering plants tend to produce only one or two inflorescences (Appendix C). Flowers vary in size with petals ranging from 4 mm to 8 mm (Figure 3.4). Inflorescences mature acropetally (i.e., from the base to top) and flowers are protogynous (i.e., stigmas become receptive, followed by anthers shedding pollen), with a ring of female flowers co-occurring above a ring of male flowers as the inforescence develops (McKone et al. 1995). Flowering occurs from mid-April to May, with peak flowering occurring from the beginning of May to mid-May for populations across the species' range (K. Chi pers. obs.). Given its early spring flowering time relative to other prairie species (Parrish and Bazzaz 1979, Runkel and Roosa 2009), *Synthyris bullii* is generally among the few species blooming at my field sites, thus minimizing any confounding effects due to simultaneously flowering heterospecifics. In late May and June, plants produce capsule fruits with winged seeds (McKone et al. 1995). In terms of conservation status, this species has been declared locally threatened or endangered in all states where it occurs (Minnesota, Iowa, Wisconsin, Illinois, Indiana, Michigan, and Ohio [extirpated]), and has been considered for federal protection (Fish and Wildlife Service 1985).

Floral Display Quantity—A total of 24 populations were surveyed across the full range of the species (Figure 3.1). Most of these populations occurred in highly fragmented agricultural and urban landscapes, and were found in locations including state parks and recreational areas, nature preserves, and properties of private citizens. My study populations occurred primarily on bare, well-drained soils (e.g., sand or gravel) in habitats historically classified as prairie and/or savanna, though sites were variable in terms of management (e.g., burning, removal of early successional woody species).

During the spring of 2010 and 2011, population size was determined by systematically surveying each study site and counting the number of plants encountered. These surveys were conducted when populations had reached peak flowering period in May. Each site was first thoroughly explored to make note of all *S. bullii* plants. Field flags were used to mark the population perimeter (i.e., the outer edges of the population where individuals still occurred). To minimize the risk of accidentally miscounting individuals, the area was then divided into 1-meter plots and each plot was carefully surveyed.

To determine the proportion of flowering individuals, I counted all flowering and non-flowering *S. bullii* plants in each population. Because most of my *S. bullii* populations occur on bare soil and/or at sites that lack active vegetation in early spring, I was confident that my surveys accurately represented the number of plants in each population. The proportion of flowering plants was calculated by dividing the number of flowering plants by the total number of plants (i.e., flowering and non-flowering individuals).

Only reproductive individuals (i.e., flowering plants) were used for my measurements of population abundance and density. Population abundance was a measure of the total number of flowering plants per population. To estimate population density, I recorded GPS coordinates around the perimeter of each population using field flags, and then mapped the area of the population using ArcGIS 10.1. The mean density of populations was calculated by dividing the number of flowering plants by the area of the population.

In addition to these traditional measurements of population abundance and density, I also considered total number of inflorescences (i.e., population inflorescence abundance) and inflorescence density per population (i.e., population inflorescence density) because pollinators may assess population resources according to the number and proximity of visible flowering

spikes rather than discrete individual plants (Schmitt 1983, Jiménez et al. 2012). The number of inflorescences produced by flowering plants was noted in the field, and these numbers were used to calculate a total number of inflorescences and inflorescence density per population.

Floral Display Quality—A total of 20 plants were randomly selected from each population for measurements of floral display at the inflorescence level. These floral display traits were measured in three ways: height of inflorescence, number of flowers, and flower density (Table 2.1). Each inflorescence was measured from its base (i.e., where the stem emerges from the ground), and I counted the total number of flowers. Flower density is a measure of the proximity between flowers on an inflorescence, and was estimated by dividing the number of flowers by the length from the bottom-most to top-most flower of the inflorescence.

Reproduction and Fitness—Reproduction was determined by measuring fruit set and seed set of the study populations. At each population, a single infructescence was randomly collected from 20 individuals. Proportion of fruit set was assessed for each infructescence by counting the total number of fruit formed and dividing this number by total flowers (i.e., fruit and unfertilized flowers). In addition, five fruits were randomly selected from each infructescence to measure seed set, which was considered the total number of seeds divided by the number of ovules produced by flowers (mean number of ovules = 52, Appendix D). Mean number of ovules was determined by randomly sampling 15 flowers from three populations, dissecting ovaries, and counting the number of ovules.

Fitness was determined by measuring seed germination. Seeds collected from each population were used to assess seed viability through germination in a growth chamber. For each population, a total of 100 seeds were counted and separated into five petri dishes lined with moistened filter paper (n = 20 seeds per dish). Dishes were wrapped in foil and placed in cold

storage at approximately 3°C for 3 months to simulate cold stratification. Seeds were then placed in a growth chamber (20°C, 14-hour photoperiod) and monitored daily during a 1-month period for evidence of germination (i.e., emergence of radicle and cotyledons).

Statistical Analyses—Prior to analysis, all data were checked for normality using QQ Plots, Box-Plots, and a calculation of the Shapiro-Wilk *W*-statistic using PROC UNIVARIATE in SAS Version 9.3 (SAS Institute 2011). All non-normal data were transformed using appropriate methods to achieve a normal distribution ($p \geq 0.05$) or a value of $W \geq 0.85$. Fruit set data were transformed using $\sin^{-1}(x)$ and germination data were transformed using $\ln(\sin^{-1}(\sqrt{x}))$. Population abundance, population inflorescence abundance, and inflorescence number per plant were transformed using $\log(x+1)$. Population density was transformed with $\log(x+0.001)$ and population inflorescence density was transformed with $\log(x)$.

Pearson's correlations (r) were used to examine relationships between floral quantity and quality (PROC CORR, SAS Institute 2011), with a $p \leq 0.05$ used to determine significance and $p \leq 0.10$ used to determine marginal significance. We analyzed the effect of floral quantity and quality on three response variables (fruit set, seed set, and germination) with general linear mixed models (GLMMs) and an information theoretic (IT) approach in R (R Development Core Team 2012). Model selection was carried out using Akaike's Information Criterion (AIC).

To minimize redundancy in the model set, I first pared down the number of potential predictors by testing for collinearity among a set of initial raw parameters (Freckleton 2010, Grueber et al. 2011). These raw parameters include all variables in Table 2.1. Using the *lmerTest* package in R (Kuznetsova et al. 2013) to generate a correlation matrix, I found strongly significant correlations ($r \geq \pm 0.75$, Kumar et al. 2006) between: (1) PopAbun and PopInfAbun (r=-0.92), (2) PopDen and PopInfDen (r=-0.76), and (3) InfDen and InfFlw (r=-0.75). We chose

to use biologically-sound reasoning to eliminate one variable from each set to minimize redundancy and false conclusions during model selection. Because pollinators may only recognize the number and proximity of flowering spikes and not discriminate between individual plants (Schmitt 1983), I judged PopInfAbun and PopInfDen were more biologically meaningful predictors than PopAbun and PopDen (respectively), and kept them in subsequent models while eliminating their counterparts. In addition, I removed InfFlw from the analysis, choosing instead to use InfDen to represent floral quality because the latter variable is more biologically interesting, and contains spatial information while InfFlw provides size information. I argue that because the variable InfHeight also contains size information, the combination of InfHeight and InfDen as predictor variables will provide sufficient spatial and size information for my analysis on floral quality.

After eliminating collinear predictors, I generated global models for the three response variables using *lmerTest* (Kuznetsova et al. 2013). The global models were developed using year as a random effect and floral quality (InfDen, InfHeight) and quantity (PopSizeInf, PopDen, PopDenInf, PropFlw) as fixed effects. Due to differences in scale within the dataset (i.e., dataset contains both proportions and integers), the global models were standardized using the *arm* package in R (Gelman et al. 2013) for a mean of zero and standard deviation of 0.5 (Gelman 2008, Grueber et al. 2011). Global models were found to converge.

Using the global models, I then derived a set of 32 submodels (i.e., a model set) for each response variable with the *MuMIn* package in R (Bartoń 2013), which computed AIC_C (Akaike's Information Criterion for small sample size), ΔAIC (i.e., the difference between a particular model vs. the best-ranked model), and w_i (i.e., Akaike weight or probability that a given model is the best of those compared) (Anderson et al. 2000, Burnham and Anderson 2002, Dauber et al.

2010). Out of the model set, the best models were selected using a criteria of $\Delta_i \leq 4$. While models of $\Delta_i \leq 2$ are thought to have substantial support, those with $\Delta_i \leq 4$ may still have some support (Burnham and Anderson 2004, Grueber et al. 2011) and were therefore considered in my analysis.

Because several models were selected and the weight of the best model for each response variable was <0.9 (Grueber et al. 2011), I used an IT model-averaging approach to account for model selection uncertainty and estimate the effects and relative importance of predictors. Using the *MuMIn* package (Bartoń 2013), I calculated the estimate (i.e., direction and magnitude of a predictor's relationship to a response variable), unconditional SE (i.e., standard error that includes model selection uncertainty), 95% confidence interval (i.e., estimate precision), and relative importance for each of the parameters in the top-ranked models (Mazerolle 2006, Grueber et al. 2011).

RESULTS

Demography—Populations ranged in size from 0 to over 3,000 flowering plants, and showed considerable fluctuation in floral quantity between years (Table 2.2). Between the two years of the study, the total number of flowering individuals and inflorescences produced by plants increased in 16 populations and decreased in eight populations. Some of the most dramatic differences were observed in CDB and NAK, which showed a nearly eight-fold increase and ten-fold decrease between years, respectively. In 2010, no flowering plants were observed in the PAZ population, though sterile individuals were found at the site.

Relationship of Inflorescence and Population Characteristics—In 2010, there was a significant positive relationship between population inflorescence abundance and flower density

(*df*=22, *r*=0.439, *p*=0.041). Inflorescence height was also found to have a marginally positive relationship with population density (*df*=22, *r*=0.359, *p*=0.101) and population inflorescence density (*df*=22, *r*=0.356, *p*=0.105) in 2010. While these results were non-significant, they nevertheless suggest that there may be a weak relationship between inflorescence size and plant density, especially because in 2011 I found a strongly significant positive relationship between inflorescence height and population inflorescence density (*df*=22, *r*=0.641, *p*=0.001). All other relationships between floral display quantity and quality were found to be non-significant (*p*>0.15).

Effects of Flower Quantity and Quality on Reproduction—Only one floral quality variable (InfDen) and quantity variable (PopInfAbun) proved to be important for the response variables I measured (Tables 1.3-1.4). However, the effect of these predictor variables was not consistent among response variables—that is, the specific predictors that explained variation and the magnitude of effects differed among response variables. The other variables (InfHeight, PopInfDen, PropFlw) were found to be poor predictors for reproductive success and fitness.

Of the 32 candidate models, variation in fruit set was best approximated by the model that contained InfDen as the only predictor, while the model that contained an interaction for InfDen and PopInfAbun was within 2 Δ_i of the best model (Table 2.3). A less conservative threshold of $\Delta_i \leq 4$ included models that contained InfHeight in addition to InfDen or PopInfAbun (Table 2.3), although there was almost no evidence supporting the model of InfHeight when it was a lone predictor (AIC_C=-0.703, Δ_i=8.486, w_i=0.01). Further, after model-averaging InfHeight was found to have 20% relative importance to InfDen and a 95% confidence interval that captured zero (Table 2.4), showing that InfHeight likely has no effect on fruit set. By

comparison, there was strong evidence that InfDen shares a positive relationship with fruit set, and PopInfAbun may also play a positive, but less important, role in affecting fruit set.

In terms of seed set, the model of PopInfAbun as a lone predictor received the strongest support (w_i=0.75, Table 2.3). In addition to being ranked the best model, it was also supported much more strongly than the second-best model (InfDen and PopInfAbun), which received considerably less support (w_i=0.10) and was close to 4 Δ_i from the top-ranked model (Table 2.3). This was also consistent with the model-averaged results, where PopInfAbun was the most important predictor followed by InfDen with a relative importance of only 12% (Table 2.4), which shows little support for InfDen as a predictor of seed set.

Similar to fruit set, germination was best explained by the model containing InfDen as the only predictor (w_i=0.46), while the third-best model (PopInfAbun) received little support (w_i=0.13, Table 2.3). In addition, the null model was found to be the second-best model in the set (w_i=0.27), suggesting that InfDen explains some of the observed variation but is not an extremely strong predictor. Further, after model-averaging, InfDen was found to have a relative importance of 54% (Table 2.4), again showing that while InfDen was the best predictor in my set and may explain some of the variation in germination, it is lacking very strong support.

DISCUSSION

The results did not provide strong support for my prediction on compensatory effects of individual size in small populations, but instead showed reproductive limitations (i.e., decreased fruit and seed set) consistent with Allee effects (Groom 1998). If compensatory effects occurred, I would have expected to find: (1) an inverse relationship between floral display size and population size, and (2) higher reproductive output and fitness associated with inflorescence size

independent of population size. Instead, I found that smaller inflorescences were associated with smaller, less dense populations. In addition, population abundance played an important role in explaining fruit and seed set for our study populations.

Interestingly, my data also showed that Allee effects could be detected only within certain measures of reproductive output. The three categories of reproduction and fitness responded differently to variables at the population versus individual level: fruit set responded to both floral quantity and quality, whereas seed set was affected by floral quantity and germination by floral quality. While floral quality and quantity act in concert to affect overall reproductive success, the importance of a specific parameter depends on the set of response variables considered—for example, seed set responded more strongly to population size than any other reproductive trait—showing that Allee effects may only manifest at specific stages during the reproductive process. Thus, detecting Allee effects may require sampling responses for a variety of reproductive processes.

Effects of Floral Display Quantity on Floral Display Quality—In my study populations, inflorescence size (i.e., flower density, inflorescence height) showed positive relationships with population abundance and density, though relationships varied by year. While some studies have found that inflorescence size can be negatively affected by density-dependent competition for resources (Mustajärvi et al. 2001, Weber and Kolb 2011), environmental changes can play a role in limiting reproduction independent of competition: populations under stress conditions, such as drought or nutrient limitation, may be unable to support large floral displays or may be confronted by tradeoffs in allocating resources to both reproductive and non-reproductive functions (Galen 1999, Galen et al. 1999, Andersson 2005). If there is a relationship between environmental shifts and floral display size, then high population inflorescence abundance may

be an indicator for suitable habitat conditions for endemic species, such as *S. bullii*, where the benefits of occurring in an optimal environment, in spite of potentially increased intraspecific competition, outweigh the disadvantages of lower quality habitat (Roll et al. 1997). These plants in large, dense populations do not suffer noticeable constraints that depress inflorescence size, reproduction, or fitness when compared to the potential environmental stress associated with small, sparse populations.

Floral Quality and Reproduction—The flower density on an inflorescence was the most important explanatory variable for fruit set and germination (Table 4). Another study on *S. bullii* also found high fruit set associated with sections of the inflorescence that had higher flower density (McKone et al. 1995). These results suggest that inflorescences with higher floral density may be more attractive for pollinators. In my study, floral density positively correlated with flower number. Larger floral displays, in terms of flower number and size, have been shown to increase pollinator visitation (Gerber 1985, Schmid-Hempel and Speiser 1988, Harder and Barrett 1995, Severns 2003, Makino et al. 2006). Reduced distance among flowers may also facilitate pollinator movement and increase the probability that a pollinator may visit multiple flowers on the same inflorescence (Pyke 1979, Mitchell et al. 2004).

Under circumstances of pollinator limitation in self-compatible species, flowers that are spatially close also have the opportunity to reproduce through geitonogamous self-pollination (Eckert and Schaefer 1998), resulting in potentially higher fruit and seed set compared to flowers that occur further apart. *S. bullii* is a self-compatible species with female-phase and male-phase flowers that open simultaneously and in close proximity (McKone et al. 1995), which potentially promotes selfing among flowers in the middle and top sections of the inflorescence, even in the absence of pollinators. However, this would likely have long-term consequences resulting in

inbreeding effects if pollinator limitation occurred over many generations. Even in self-compatible species, geitonogamous selfing can have serious consequences for offspring fitness in terms of inbreeding depression (de Jong et al. 1993, Husband and Schemske 1996).

While inflorescence size served as an important explanatory variable for fruit set, it failed to have a strong relationship with seed set. Moreover, I failed to detect an inverse relationship between population abundance and inflorescence size—that is, I did not find larger floral displays associated with smaller, less dense populations. If floral quality could explain reproductive success at all stages independent of population quantity, and if smaller populations were found to produce larger floral displays, then my results would have supported the prediction of compensatory effects of plant size (i.e., large plants experience no loss of reproduction in small, sparse populations). My results showed that larger inflorescences in small populations did not experience the same success in terms of seed production compared to plants in large populations.

Floral Quantity and Reproduction—Variation in seed set was best explained by population inflorescence abundance: as the abundance of flowering stalks increased, there was a corresponding positive effect on seed set. The observed trend could be potentially explained by the observed differences in pollinator and pollen limitation in small vs. large plant populations. In the absence of pollinators, small populations of self-compatible, protogynous plants, such as my study species, are predicted to reproduce more frequently by geitonogamous selfing (de Jong et al. 1993, Ågren 1996). Such autonomous processes likely lead to reduced pollen quality and quantity that have negative consequences for seed set (Aizen and Harder 2007). Even among self-compatible plants, reproduction via self-pollination has been found to result in a 20% decrease of seed set on average (Husband and Schemske 1996), due to pollen quality and the

expression of deleterious alleles in selfed embryos (Aizen and Harder 2007). Thus, reduced seed set in small populations may be caused by a combination of pollinator limitation and the plant's breeding system.

When examining the results, I also observed that, interestingly, only seed set showed a strong positive response to population inflorescence abundance—fruit set responded weakly and seed germination showed no relationship with population size. The results of my present study offer a comparison between fruit set and seed set in response to population abundance; specifically, the different responses in fruit vs. seed set may reflect differing sensitivities to plant abundance and its related impacts. This observed difference may be explained by pollen limitation associated with small population size. Modest floral resources in small populations are predicted to be inadequate for attracting pollinators (Waites and Ågren 2004)—these small populations thus suffer from cascading Allee effects in the form of reduced pollinator visitation leading to decreased pollen receipt and subsequently reduced fruit and/or seed set (Ågren 1996, Groom 1998). The impact of population size on seed set is well-studied (Severns 2003, Knight et al. 2005), and these previous studies show that seed set can indeed be extremely sensitive to reduced pollen quantity and quality associated with small populations (Young 1997, Waites and Ågren 2004). In terms of the comparative effect in fruit set vs. seed set, the variables are predicted to show similar negative responses to pollen limitation in small populations (Knight et al. 2005). However, there does appear to be some evidence for differences in magnitude of response of fruit set relative to seed set among species (Burd 1994). The potential differences in pollen requirements that trigger these two processes (i.e., pollen limitation may operate at different thresholds for fruit vs. seed formation) can thus lead to different responses in fruit vs. seed set when pollen limitation is associated with population size. Future studies should

explicitly test for pollen limitation in *S. bullii* through pollen-augmentation experiments to determine if small populations for this species do suffer from pollen limitation, and if there are true differences in the level of pollen limitation experienced for fruit vs. seed set.

Another potential explanation for the observed positive relationship between seed set and population inflorescence abundance could be the increased inbreeding depression associated with small populations. Inbred individuals are more likely to suffer from decreased reproductive output, including lower seed set (Keller and Waller 2002, Severns 2003). Populations that have been isolated for many generations have a potential for purging deleterious alleles, but this is less likely for perennial, long-lived species (Byers and Waller 1999), especially for alleles that are only mildly deleterious (Husband and Schemske 1996). Moreover, studies have found that inbreeding effects are still manifesting in populations of many North American species (e.g., Leimu et al. 2006), even for populations that have been isolated for decades (Berry et al. 2013). A future study on the population genetics of *S. bullii* could determine whether decreased seed set in small populations is associated with inadequate pollen quantity/quality, inbreeding depression, or both.

I failed to detect a relationship between germination and population inflorescence abundance in the current study, indicating that population size potentially has little influence on this aspect of fitness. Population inflorescence abundance appears to impose limitations on the reproductive stage (i.e., seed set), but not recruitment (i.e., seed viability). However, my seed germination experiments were conducted under ideal laboratory conditions, and past research suggests that this may not be a reliable method for assessing true fitness consequences of population size because it removes the environmental context under which these seeds would normally germinate (Ramsey and Vaughton 1998, Morgan 2001). As my results with fruit and

seed set show, the impacts of population size on fitness may manifest at different stages of reproduction—therefore, even when seed viability is high under ideal conditions, problems could still manifest at other stages of recruitment.

Conclusion—This study provides an example of Allee effects by showing that population abundance has a positive impact on floral display quality and reproduction in a rare perennial species. Specifically, large, dense populations had larger inflorescences, increased reproductive output, and higher seed viability, showing no reproductive tradeoffs for the variables measured. There was a strong relationship between reproductive success and population abundance, while inflorescence size played an important role in fruit set and may potentially compensate for reproductive challenges in small populations, floral quantity was the most important factor in determining seed production. More interestingly, these results show that floral quality and quantity affect reproduction at different stages, but ultimately a combination of the two factors are necessary for maximizing reproduction in rare species.

These results are especially relevant to endangered species management and ensuring the viability of rare populations. Because of the complex relationships between floral quantity, floral quality, and reproduction, management plans that aim to maximize population size may also result in cascading effects that also improve floral display and reproductive success. Future studies should examine other factors that could be influencing population abundance/density and reproductive success, such as habitat quality. These studies could determine if plants in the study populations suffer from competition or environmental stress. In addition, future work should also specifically address potential pollination and pollen limitation for this system, as it could be a large factor that helps explain the impacts of population size on reproduction and recruitment.

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TABLES AND FIGURES

Table 2.1. Traits used to characterize floral quality and quantity at the individual-level and the population-level for all 24 populations of *Synthyris bullii*. Using combinations of these traits, a set of 49 candidate models were developed a priori to explain reproductive output and fitness in study populations.

Floral Display Trait	Abbreviation	Definition
Floral Quality (Individuals)		
Flower Density	InfDen	Mean number of flowers per cm^2 of an inflorescence
Inflorescence Height	InfHeight	Length from base of stem to the top-most flower of inflorescence
Flower Abundance	InfFlw	Total number of flowers on an inflorescence
Floral Quantity (Population)		
Proportion Flowering	PropFlw	Proportion of flowering plants relative to the total number of plants (flowering and non-flowering) in a population
Population Abundance	PopAbun	Total number of flowering plants in a population
Population Inflorescence Abundance	PopInfAbun	Total number of inflorescences for all flowering plants in a population
Population Density	PopDen	Mean number of flowering plants per m^2 in a population
Population Inflorescence Density	PopInfDen	Mean number of inflorescences for all flowering plants per m^2 in a population

Table 2.2. Study populations of *Synthyris bullii* in 2010 and 2011. Measurements of population size and density were recorded at the level of individual plants and inflorescences.

Site	Area (m^2)	Proportion Flowering		Population Abundance		Population Inf. Abun.		Population Density		Population Inf. Density	
		2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
LMF	215.8	20.33	20.48	158	127	196	160	0.73	0.59	0.91	0.74
LMR	135.0	36.60	36.19	220	186	274	245	1.63	1.38	2.03	1.81
NAK	1212.0	26.67	8.16	708	70	1050	73	0.58	0.06	0.87	0.06
NAT	57.0	19.70	51.11	66	69	84	119	1.16	1.21	1.47	2.08
FUL	1180.6	1.52	6.55	23	35	24	36	0.02	0.03	0.02	0.03
HAH	29.2	39.79	37.76	78	54	97	66	2.67	1.85	3.32	2.26
PAO	287.5	9.42	50.57	18	89	19	123	0.06	0.31	0.07	0.43
PAZ	53.1	0	30.95	0	13	0	15	0	0.25	0	0.28
PAM	1222.2	26.08	39.29	42	55	59	82	0.03	0.05	0.05	0.07
EGC	5421.7	24.28	26.04	287	87	346	125	0.05	0.02	0.06	12.36
GRI	5.19	28.33	14.71	17	5	20	6	3.28	0.96	3.85	1.16
ADS	4424.2	54.35	50.27	75	95	142	170	0.29	0.37	0.56	0.67
CDB	3638.9	15.98	57.36	371	3114	486	6942	0.10	0.86	0.13	1.91
RTR	1016.9	20.25	41.31	113	725	164	946	0.11	0.71	0.16	0.93
CPT	345.8	18.38	36.30	25	53	38	73	0.07	0.15	0.11	0.21
WCR	11.8	33.33	12.9	13	4	21	5	1.10	0.34	1.78	0.42
THN	63.4	1.79	84.62	1	11	1	28	0.01	0.17	0.01	0.44
ACW	12.3	66.67	25.81	22	8	60	12	1.79	0.65	4.88	0.98
FOS	39.4	40.00	50.91	18	28	29	48	0.46	0.71	0.74	1.22
STC	158.2	11.25	35.13	18	111	23	149	0.11	0.70	0.15	0.94
ALX	254.4	54.35	50.27	75	95	142	170	0.29	0.37	0.56	0.67
MUB	51.2	20.29	33.33	71	74	79	97	1.39	1.44	1.54	1.89
TCH	245.8	1.79	17.14	1	12	1	19	0.01	0.05	0.01	0.07
LKW	26.6	20.00	25.97	25	40	38	50	0.94	1.51	1.43	1.88

Table 2.3. Candidate models were generated to explain reproduction in *Synthyris bullii*. Shown is the summary of model comparisons using AIC_c (Akaike's Information Criterion for small sample size), Δ_i (difference in AIC between a given model and the best model), and w_i (Akaike weight or the probability that a given model is the best model in a set). Presented are the global model, null model, and candidate models found to have strong ($\Delta AIC \leq 2$) or moderate strong ($\Delta AIC \leq 4$) support in the model set.

Model	***df***	**AICc**	Δ_i	w_i
Fruit Set				
Global	8	7.995	17.184	7.06e-05
Null	3	9.938	19.127	2.67e-05
InfDen	4	-9.189	0	0.38
InfDen x PopInfAbun	5	-8.867	0.322	0.32
InfDen x InfHeight	5	-6.846	2.343	0.12
InfDen x InfHeight x PopInfAbun	6	-5.485	3.704	0.06
Seed Set				
Global	8	-103.836	27.546	7.77e-07
Null	3	-119.099	12.283	1.60e-03
PopInfAbun	4	-131.382	0	0.75
InfDen x PopInfAbun	5	-127.446	3.936	0.10
Germination				
Global	8	-19.037	25.023	1.68e-06
Null	3	-42.987	1.073	0.27
InfDen	4	-44.060	0	0.46
PopInfAbun	3	-41.490	2.570	0.13

Table 2.4. Relative effects of predictors on fruit set, seed set, and germination for *Synthyris bullii* after model-averaging. Only predictor variables in top-ranked models ($\Delta AIC \leq 4$) are shown. Estimate is a regression (R^2) estimate of the effect of a given predictor on a given response variable. Unconditional standard error (Uncond. SE) and confidence intervals (95% CI) show the precision of the estimate. Relative Importance estimates the importance of a given predictor relative to the most important predictor in a set.

Parameter	Estimate*	Uncond. SE	95% CI	Relative Import.
Fruit Set				
(Intercept)	0.817	0.063	(0.694, 0.940)	
InfDen	0.270	0.067	(0.139, 0.401)	1.00
InfHeight	0.132	0.068	(-0.001, 0.265)	0.20
PopInfAbun	0.139	0.055	(0.031, 0.247)	0.43
Seed Set				
(Intercept)	0.175	0.010	(0.155, 0.196)	
InfDen	0.035	0.015	(0.006, 0.064)	0.12
PopInfAbun	0.073	0.015	(0.043, 0.102)	1.00
Germination				
(Intercept)	0.258	0.019	(0.221, 0.294)	
InfDen	0.107	0.036	(0.037, 0.178)	0.54
PopInfAbun	0.093	0.038	(0.017, 0.168)	0.15

*Effect sizes are standardized using 2 SD (Gelman 2008, Grueber et al. 2011).

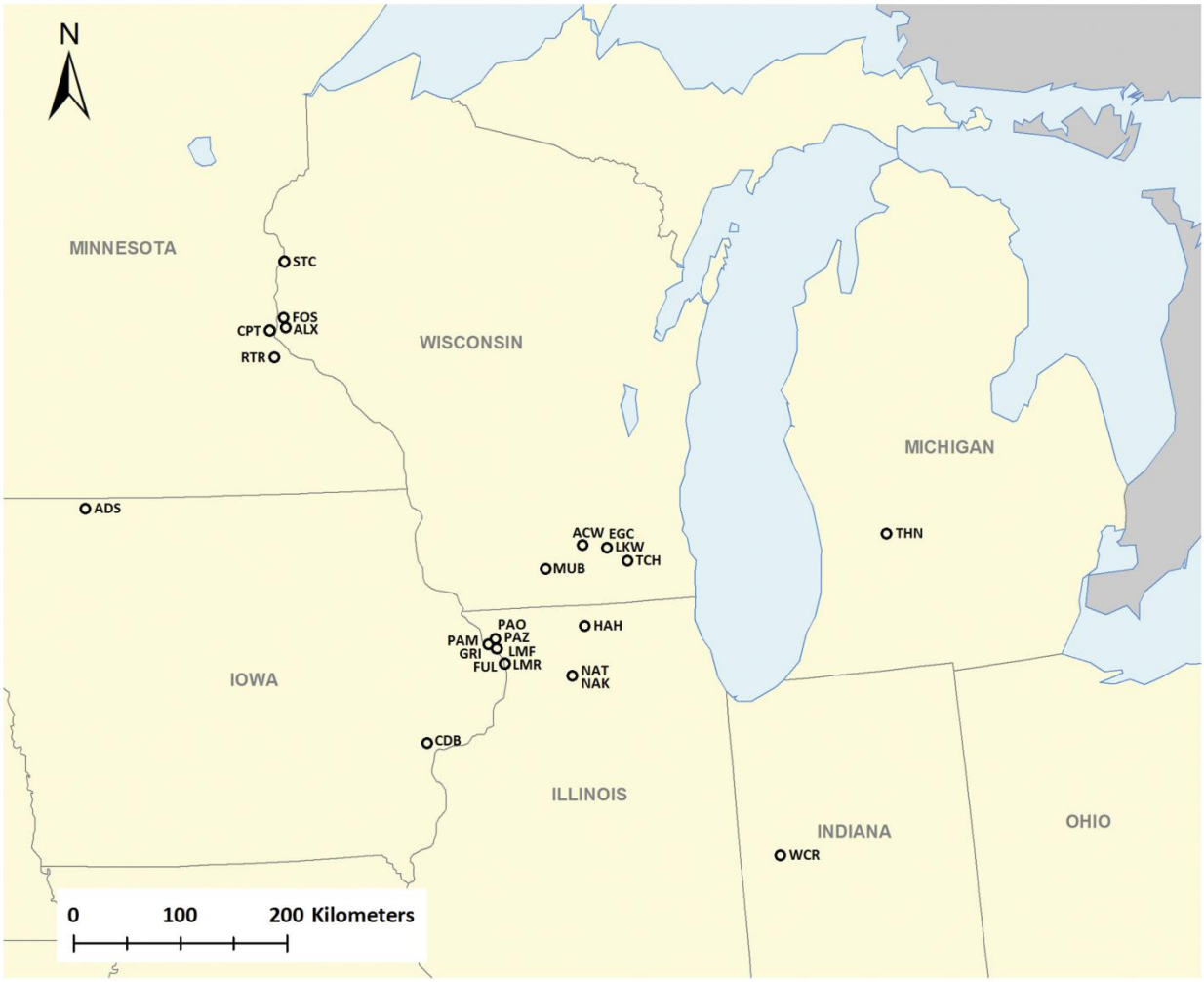

Figure 2.1. Map showing locations of *Synthyris bullii* populations used in this study. *S. bullii* is endemic to the Midwestern United States, and sites were chosen to represent the species' range.

CHAPTER 3

REPRODUCTIVE MORPHOLOGY ASSOCIATED WITH HABITAT DEGRADATION IN PRAIRIES OF THE MIDWESTERN UNITED STATES

ABSTRACT

Premise of the Study. In North America, prairie and savanna remnants suffer from increased habitat degradation and structural changes (e.g., woody encroachment). Plant populations in remnant habitats may be threatened over time as a result of this degradation. Past studies have explored plant responses to such changes by using common species and focusing primarily on vegetative characteristics rather than reproductive ones. Our research uses a rare Midwestern endemic, *Synthyris bullii*, to determine if morphology of reproductive structures are associated with habitat quality.

Methods. Study populations were visited and categorized according to the level of observed woody encroachment: shaded (N=3), semi-shaded (N=2), and open (N=2). From each population, I randomly selected 20 individuals to measure inflorescence size (e.g., inflorescence height, flower density, flower number) and flower size (e.g., lengths of petals, stamens, carpels). Data was explored by canonical discriminant analysis to determine if traits associated with particular habitat types, then analyzed by MANOVA and univariate ANOVAs to determine differences in traits among habitats.

Key Results. Our results showed that particular morphologies were associated with open and shaded habitats, though semi-shaded habitats did not generally differ from the other two categories. Specifically, shaded habitats produced smaller inflorescences (e.g., decreased flower

density and flower number) with larger flowers (e.g., increased lengths of petals and carpels), while the inverse pattern was observed in open habitats.

Conclusions. While I initially predicted consistent reductions in size across reproductive traits, I instead found tradeoffs in inflorescence and flower size associated with different habitats. Changes in the relative sizes of reproductive traits may have implications for the breeding systems of populations in different habitats. For example, within shaded habitats, elongation of carpels in flowers may increase selfing rates. These results are consistent with past research that has found that populations shift to favor selfing under resource scarcity.

INTRODUCTION

Widespread land-use change, such as urbanization and the introduction of industrialized agricultural practices, has led to the destruction and degradation of natural habitat in many areas of the world (Howe 1994, Samson and Knopf 1994, Samson et al. 2004). Specifically in North America, native prairies have become increasingly degraded due to the disruption of historic fire patterns, leading to encroachment by early successional woody species (Bragg and Hulbert 1976, Leach and Givnish 1996, Briggs et al. 2002). Establishment of woody species can result in significant modifications to local microenvironments, including changes to light availability and soil chemistry (Ratajczak et al. 2012), which can subsequently exclude sensitive prairie and savanna species that are not adapted to such conditions. Woody encroachment thus acts as a threat to native biodiversity, and its effects on prairie species should be carefully considered.

Plants are well known for displaying highly variable phenotypic responses as a result of environmental conditions (Sultan 2000, Valladares and Niinemets 2008), and therefore might be expected to respond to localized habitat changes associated with woody encroachment. Indeed,

the alteration of light patterns has been found to considerably alter growth, development, and biomass allocation of herbaceous species (Slade and Hutchings 1987, Stuefer and Huber 1998, Weijschedé et al. 2006). However, compared to studies on the vegetative responses of plants, research on the effect of environmental variation on reproductive traits has been more limited (Benvenuti et al. 1994, Li et al. 2001), or has been restricted primarily to economically important species (Harris and Scott 1969, Zhao et al. 2012).

Moreover, past studies that have explored reproductive responses to environmental variation have focused primarily on life history traits involved with development and the timing of reproduction (Pigliucci 1997, Sultan 2000, Harder and Johnson 2005). For example, an extensive body of research has developed in areas such as "reproductive assurance" strategies, in which some species are able to switch from outcrossing to selfing as a response to mate availability (Vogler et al. 1998, Tsitrone et al. 2003). In terms of responses to variation in the abiotic environment, previous studies show that some species can control the type of flowers they produce (i.e., chasmosgamous vs. cleistogamous) as consequence of light and nutrient availability (Le Corff 1993, Mattila and Salonen 1995, Culley 2002). Ultimately, much of the research on life history traits and environmental variation show that plants favor selfing strategies while under resource scarcity or unfavorable environmental conditions.

While plasticity in life history traits has important implications for plant reproduction, fewer studies have explored changes in reproductive morphology, specifically variation in size of reproductive organs. However, variation in floral display size may have important implications for reproductive success and subsequent population viability. Increased shading and its microclimatic effects (e.g., decreased temperature, decreased light availability) have been previously found to reduce flower size (Harris and Scott 1969). Pollinators preferentially visit

plants with larger, more impressive floral displays (Conner and Rush 1996, Grindeland et al. 2005), potentially because flower size may serve as an indicator of resource rewards available to pollinators (Fenster et al. 2006). Because the ability of a plant to attract insect pollinators may depend on the size of its floral display, reductions in inflorescence or flower size associated with habitat change could compromise a plant's reproductive potential.

Such issues may be of particular concern in rare species. Many rare species are thought to tolerate only a narrow range of environmental conditions (Rabinowitz 1981), as evidenced by their rapid loss in habitats undergoing degradation (Clark & Tilman 2008, Klejin et al. 2008). Any habitat degradation that results in morphological changes, especially in terms of reproductive organs, is a significant conservation concern as these changes may have associated fitness costs that contribute to their decline.

Our study aims to address the following question: does habitat quality affect reproductive morphology in a rare species? In particular, I was interested in determining if degraded habitats were associated with morphologies that would be unfavorable for attracting pollinators (e.g., reduced flower size, reduced flower number per inflorescence). The reduction of floral attractiveness may also be associated with increased selfing and/or a selfing strategy in response to changes in environmental conditions, specifically an increase in shading. I defined habitat quality in terms of the amount of woody encroachment occurring at a particular population, and I considered reproductive structure at two levels: the inflorescence and the flower.

MATERIALS AND METHODS

Study Species—To address my research question, I used the rare species *Synthyris bullii* (Eaton) A. Heller (Plantaginaceae, Kittentail, synonym *Besseya bullii* (Eaton) Rydb.

[*Scrophulariaceae*]). The species' prominent characteristics include a large basal rosette (Gleason and Cronquist 1991), and one or more spiked inflorescences (K. Chi pers. obs.). Inflorescences mature acropetally (i.e., flowering begins at the base and proceeds upward) and flowers are protogynous (i.e., stigmas are receptive for a period of time before pollen is shed, McKone et al. 1995). *Synthyris bullii* has also been determined to be self-compatible and pollinated primarily by bees (McKone et al. 1995).

Synthyris bullii is endemic to gravel and sand prairie-savannas of the Midwestern United States (Gleason and Cronquist 1991). This species was previously considered for federal protection (Fish & Wildlife 1985) and is currently listed as threatened or endangered in all states across its range (Minnesota, Iowa, Wisconsin, Illinois, Indiana, Michigan, and Ohio [extirpated]). Anecdotal accounts suggest that habitat degradation is a potential cause of decline for this species (K. Chi pers. obs.). Indeed, decreased reproductive output (e.g., fruit set, seed set) has been observed in populations where woody encroachment occurs (Figure 4.2).

Study Sites and Sampling—Because my study sought to address the effect of woody encroachment on floral traits, I selected seven study populations where the local habitat could be clearly assigned to one of three categories: open, semi-shaded, or shaded (Table 3.1). Open habitats included prairies, which were dominated by short, herbaceous vegetation and no shading from an above woody canopy. Semi-shaded habitats represented an intermediate category where herbaceous plants were still the dominant vegetation, but the *S. bullii* population occurred in at least partial shade as a result of one or two trees in the immediate area. The final category consisted of shaded habitats: areas where woody vegetation formed a closed canopy and very little sky was visible above the population. Habitat categories were found to be associated with different canopy cover (Appendix E) and soil characteristics (Appendix F). To minimize the

influence of geographical factors, all study sites were selected from Illinois at a similar latitude and elevation.

During the beginning of flowering in late April of 2011, 20 individuals were haphazardly chosen from each population by tossing a ball blindly and tagging the individual closet to the ball's landing position. These plants were used to collect measurements of inflorescence size and flower size. Because there may be a potential relationship between inflorescence number and flower size (Worley and Barrett 1991), I attempted to minimize the confounding effects of inflorescence number by only sampling individuals with a single inflorescence.

Populations were visited once per week to check flowering progress. From each plant, I then collected flowers to be dissected and used for floral morphometrics. A flower's location on an inflorescence can influence its size (Ashman and Hitchens 2000), thus I attempted to control for position effect by only sampling flowers from the middle of the inflorescence. Flowers were considered suitable for collection during the transition phase when the stigma was no longer receptive and anthers had just begun to shed pollen. All collected flowers were preserved in formalin-acetic-alcohol (FAA) prior to dissection. At the end of flowering, the 20 selected inflorescences were collected to assess inflorescence size.

Inflorescence Size—For each inflorescence, I collected three measurements: inflorescence height, flower number, and flower density. Inflorescence height was the total length of an inflorescence from the base of the stem to the top-most flower of the inflorescence. To determine flower number, I counted the total number of flowers on each inflorescence, using a black marker to lightly mark each flower to avoid accidentally counting the same flower more than once. Flower density was used to approximate the spatial proximity of flowers on an

inflorescence, and was measured by dividing the total number of flowers by the length from the bottom-most flower to the top-most flower.

Floral Morphometrics—Flowers were preserved in FAA solution at room-temperature for six months before dissection. Floral traits were measured by placing each flower under a dissecting microscope at 10x magnification (Fig. 1a), rehydrating the specimen with distilled water, and carefully separating the floral structures with fine-point forceps. I specifically measured the lengths of these traits: posterior petal lobe, anterior petal lobe, sepal, pistil, and stamen (Fig. 1b). Hufford (1995) identified *S. bullii* as having tetramerous corollas (i.e., having four lobes): two lateral lobes, a shorter anterior lobe, and a longer posterior lobe. For each flower, the corolla was first separated from the calyx and split at the sinus between the lateral lobe and posterior lobe (Hufford 1995). I estimated corolla size using both the anterior lobe and posterior lobe by measuring the length from the base of the corolla to the tip of the lobe. Because sepal size in this species can vary depending on position (Hufford 1995), the shortest abaxial sepal was selected from each flower and measured from the base to the tip. The length of the pistil was measured from the base of the ovary to the top of the stigma, and the stamen was measured from the base of the filament (i.e., the location where it separated from the corolla) to the top of the anther.

Data Analysis—Prior to analysis, all data were checked for multivariate normality using Q-Q Plots, Mahalanobis distances, and calculation of the Shapiro-Wilk *W*-statistic using the package *mvnormtest* in R software (Jarek 2013, R Development Core Team 2012). Our first attempt to normalize the inflorescence and flower morphometric data involved numerous transformations, but no method could achieve normality. Outliers were then identified using Mahalanobis distances and the command *mvoutlier* with an alpha=0.10, resulting in the deletion

of: (1) six data points out of 127 samples for the inflorescence dataset, and (2) four data points out of 94 samples for the floral morphometric dataset. The inflorescence dataset was found to be normal after removing outliers (W=0.982, P=0.109). After removal of data points and log-transformation, the floral morphometric dataset appeared approximately normal when examining the Q-Q Plot. I also tested my data for homogeneity of variance using Levene's test in the *car* package with habitat as a factor (Fox et al. 2013). Data for all inflorescence and floral traits met the assumption of homogeneity of variance (P>0.05).

I explored the data using canonical discriminant analysis in the *candisc* package (Friendly and Fox 2013). Canonical discriminant analysis is a statistical approach that uses quantitative traits to maximally separate samples into groups while minimizing variation within groups (Cruz-Castillo et al. 1994). This technique determines the canonical variable (i.e., linear combination of traits) that best predicts habitat categories for the given samples. Moreover, this analysis has been shown to be robust with data that do not necessarily meet the assumption of multivariate normality (Lachenbruch and Goldstein 1979, Cruz-Castillo et al. 1994).

Package *candisc* was used to generate discriminant functions, eigenvalues (i.e., the explanatory power of a given function), standardized canonical coefficients (i.e., the relative contribution of each independent variable in a discriminant function), structure coefficients (i.e., the relationship between each independent variable and the discriminant function), and basic canonical plots. For the analysis of inflorescence characteristics, I used the independent variables: inflorescence height (InfHeight), total number of flowers (FlwTot), and flower density (FlwDen). The independent variables used for analyzing flower traits were the lengths of: anterior corolla lobe (APetalLn), posterior corolla lobe (PPetalLn), sepal (SepalLn), stamen (StamenLn), carpel (CarpelLn), and style (StyleLn).

Next, I examined individual inflorescence and flower traits, and determined if these traits differed among habitat types. Because morphological characters are often correlated and can result in misleading interpretations of data (Willig et al. 1986), I first analyzed the inflorescence and flower datasets using multivariate analysis of variance (MANOVA). Similar to canonical discriminant analysis, MANOVA has been shown to be robust against mild violations of normality (Mardia 1971, Gupta et al. 2008). Analyses for the two sets of traits (e.g., inflorescence and flower) were run using package *car* (Fox et al. 2013). After obtaining a significant result for my MANOVA test ($P \leq 0.05$), I proceeded to examine each variable separately using ANOVA tests, followed with *post hoc* Tukey test comparisons.

RESULTS

Inflorescence Traits and Habitat—Results of the canonical discriminate analysis showed that CDF_1 was found to explain 97.717% of the variation in inflorescence traits for the three habitat types (Fig. 3.2a). Moreover, CDF_1 was found to be significant (Wilks Lambda=0.721, F=10.389, P<0.001). By comparison, CDF_2 was non-significant, and could only explain 2.283% of variation in inflorescence traits (Fig. 3.2a). Of the three traits examined, flower density was found to be the most important variable for discriminating habitat types, with inflorescence height and total flowers having a much weaker contribution (Table 3.2).

Inflorescence traits differed significantly among the three habitat types (MANOVA, Wilks Lambda=0.721, F=6.867, P<0.001). Univariate tests showed that there were significant differences among habitat types for inflorescence height (F=3.307, P=0.040), flower number (F=20.354, P<0.001, Fig. 3.3b), and flower density (F=13.975, P<0.001, Fig. 3.3c).

In terms of inflorescence height, inflorescences from open and shaded habitats differed significantly (Tukey test, P=0.031), but there was no difference between semi-shaded habitats and the other two categories (Fig. 3.3a). For flower density and flower number, open habitats were found to differ from shaded and semi-shaded habitats (Tukey test, P<0.001, Fig. 3.3b-c), but semi-shaded and shaded habitats did not differ.

Flower Traits and Habitat—In terms of flower traits, CDF_1 could explain 82.935% of variation while CDF_2 explained 17.065% of variation (Fig. 3.2b). Additionally, both CDF_1 (Wilks Lambda=0.550, F=14.994, P<0.001) and CDF_2 (Wilks Lambda=0.888, F=11.005, P=0.001) were found to be significant. In CDF_1, the lengths of the sepal, posterior petal lobe, and style were the primary traits driving the separation among groups (Table 3.2). By comparison, CDF_2 used the lengths of the anterior petal lobe and carpel as the primary variables for discriminating among groups (Table 3.2).

Flower traits were found to differ significantly among habitat types (MANOVA, Wilks Lambda=0.550, F=4.766, P<0.001). While stamen length did not differ significantly among habitat types, univariate tests showed that measurements differed for all other traits when comparing habitats: anterior petal lobe (F=6.073, P=0.003, Fig. 3.4a), posterior petal lobe (F=9.624, P<0.001, Fig. 3.4b), sepal (F=14.342, P<0.001, Fig. 3.4c), carpel (F=6.772, P=0.002, Fig. 3.4e), and style (F=9.785, P<0.001, Fig. 3.4f).

Tukey *post hoc* tests showed that flowers collected from open habitats, when compared to shaded and semi-shaded habitats, were significantly smaller in terms of the length of the anterior petal lobe, posterior petal lobe, carpel, and style (Tukey test, $P \leq 0.05$, Fig. 3.4). However, I found no differences in sizes when comparing shaded and semi-shaded habitats for these traits. All

habitat types differed significantly in terms of sepal length (Tukey test, $P \leq 0.05$, Fig. 3.4). In addition, there were no significant differences among habitats for stamen length.

DISCUSSION

My results showed that there were distinct morphologies associated with open versus shaded habitats. For the traits measured, plants from semi-shaded and shaded habitats appeared similar in terms of the sizes of their inflorescences and flowers. While I initially predicted that reproductive structures within a particular habitat might exhibit consistent changes across traits (i.e., decreases in both inflorescence and flower size associated with shading), my results instead suggest that there may be a tradeoff between inflorescence size and flower size.

Historically, floral traits were thought to have a strong genetic basis (Berg 1960, Conner and Via 1993, Pélabon et al. 2011); that is, reproductive traits should show little phenotypic variability under differing environmental conditions compared to vegetative ones. This canalization of reproductive traits is thought to occur because the arrangement and shape of flowers has evolved to maximize pollination from specific animal mutualists (Berg 1960, Galen and Cuba 2001). However, my results show that the size of reproductive traits were associated with specific habitat types and could potentially vary as a response to the level of woody encroachment and environmental changes associated with encroachment.

While my study did not address the underlying genetics that may contribute to these morphological differences among habitats, experimental research has shown that the size of floral structures can vary in response to the ratio of red to far-red (R:FR) light due to plasticity in some reproductive genotypes (Weinig 2002, Brock and Weinig 2007). Under shade conditions (i.e., low R:FR), plants may also potentially experience a tradeoff in vegetative and reproductive

traits, where elongation of petioles as a shade-avoidance strategy may impose costs on floral display size (Brock and Weinig 2007, Brock et al. 2010). Thus, one might reasonably predict a reduction in floral traits for plants grown under shade conditions.

However, in my results, I failed to observe reduced sizes of floral traits in shaded habitats. Rather, I observed an increase in many flower features (e.g., mean lengths of petals and carpels) compared to plants from open habitats. Increased carpel size for this species may have important implications for its breeding system. In my study populations, the elongated carpels in shaded habitats were more equivalent in length with stamens. This reduction in herkogamy (i.e., spatial distance between stigma and anther) is often associated with increased selfing (Lloyd 1992, Karron et al. 1997). Past research shows that reduced herkogamy can occur in populations under unfavorable environmental conditions (Elle and Hare 2002, Levin 2010), perhaps to increase the probability for reproductive success when resources may be limiting. In addition to modifications to the abiotic environment, shading can also result in decreased visitation by insect pollinators—as exothermic organisms, insects are sensitive to microclimatic changes and may avoid shaded areas (Herrera 1995, Culley 2002, Cortes-Palomec and Ballard 2006, Kilkenny and Galloway 2008). Indeed, pollinator visitation for this species does appear to be reduced in shaded habitats (Figure 4.2). Reduced insect pollination in encroached areas may subsequently favor decreased herkogamy and increased selfing in plants.

Interestingly, the reduced sizes of some floral features in open habitats were accompanied by increases at the inflorescence level, specifically in terms of flower density and flower number. The reverse pattern (i.e., reduced inflorescence size and increase flower size) was also observed in shaded habitats. This particular relationship may represent a tradeoff in the allocation of resources for reproduction, where an individual is unable to simultaneously maximize both

flower size and number of flowers (Worley and Barrett 2000, Worley et al. 2000, Sargent et al. 2007). As previously mentioned, the elongation of carpels in encroached habitats may be a strategy to reduce herkogamy and increase selfing under resource scarcity, and this increase in flower size may come at the cost of producing more flowers. Moreover, seed production is energetically expensive compared to increasing flower size; thus, under resource scarcity, plants may opt to increase flower attractiveness rather than increasing flower number. Our previous findings for this species show that reproductive success (i.e., fruit set) is positively correlated with flower density (Chi and Molano-Flores 2014). Therefore, reductions in flower density to optimize flower size may still result in overall reduced reproductive output for populations in encroached habitats.

While my research merely shows the association between reproductive morphology and encroachment, these results nevertheless take important steps towards understanding the impact that habitat degradation may have on the reproductive biology of rare plant species. Specifically, I observed a tradeoff between flower size and inflorescence size for plants growing in different habitats, and the change in inflorescence size in increasingly encroached areas may represent changes within the reproductive strategies of populations. An important future direction for this research would address the genetic basis of these observed differences among reproductive traits in plants found under encroached conditions. A reciprocal transplant experiment or manipulation of shading (e.g., building shade canopies above plants in open habitats, removing woody vegetation in shaded habitats) may resolve this issue, though such approaches should be used with caution due to the sensitivity of rare species. Examining the underlying genetics of this system would shed light on the degree to which the observed morphological differences are a

result of plastic responses to resource scarcity versus traits that may be undergoing selection after several years of habitat degradation.

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TABLES AND FIGURES

Table 3.1. Number of *Synthyris bullii* populations used for assessing the association between morphological traits and habitat quality. Populations were assigned to a habitat type based on the canopy cover above each population.

Habitat Type	Number of Sites	Characteristics
Open	2	No woody vegetation present; dominated by herbaceous vegetation; no canopy cover
Semi-Shaded	2	Some woody vegetation present (<5 trees/shrubs); population is partially shaded
Shaded	3	Woody vegetation is dominant growth form; canopy is completely closed above

Table 3.2. Summary statistics for standardized canonical coefficients (SCC) and structure coefficients (*r*) for two canonical discriminant functions (CDF). The independent variables are measurements of morphological traits at two levels (inflorescence and flower) for the species *Synthyris bullii*.

	CDF_1		CDF_2	
Variable	SCC	*r*	SCC	*r*
Inflorescence Traits				
InfHeight	-0.203	0.424	2.001	-0.689
FlwTot	0.157	0.833	2.941	0.511
FlwDen	0.992	0.969	-3.200	-0.202
Canonical Correlation	0.273		0.009	
Eigenvalue	0.375		0.009	
Variation Explained (%)	97.717		2.283	
Flower Traits				
APetalLn	-0.114	0.460	0.431	0.611
PPetalLn	0.227	0.643	0.263	0.460
Sepal	0.757	0.796	-0.294	0.250
Stamen	-0.240	0.129	0.157	0.495
Carpel	-1.132	0.484	1.941	0.637
Style	1.490	0.662	-1.582	0.387
Canonical Correlation	0.381		0.112	
Eigenvalue	0.615		0.126	
Variation Explained (%)	82.935		17.065	

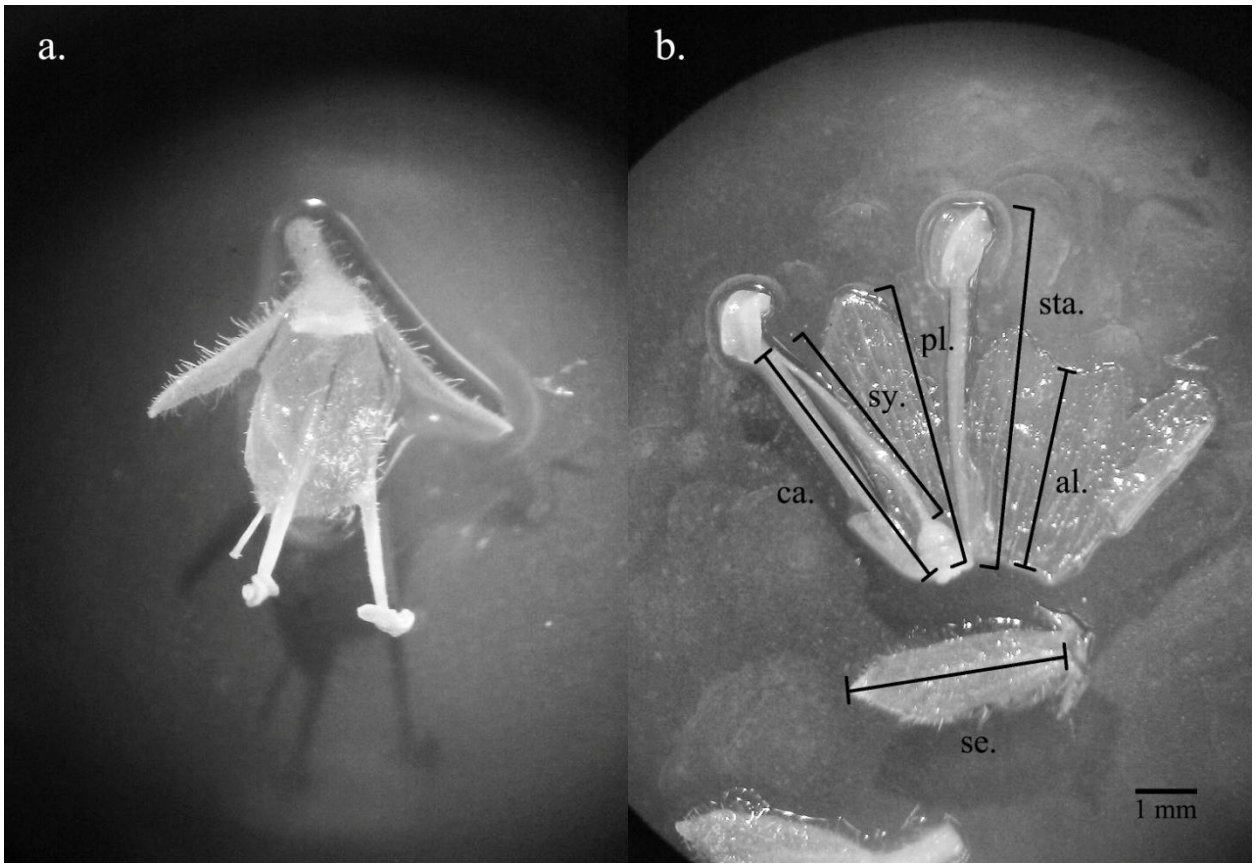

Figure 3.1. (a) Intact flower of *Synthyris bullii* under dissection microscope at 10x magnification. (b) Flower dissected and flattened to show floral parts that were measured. ***pl*** = posterior petal lobe, ***al*** = anterior petal lobe, ***se*** = sepal, ***sta*** = stamen ***ca*** = carpel, ***sy*** = style.

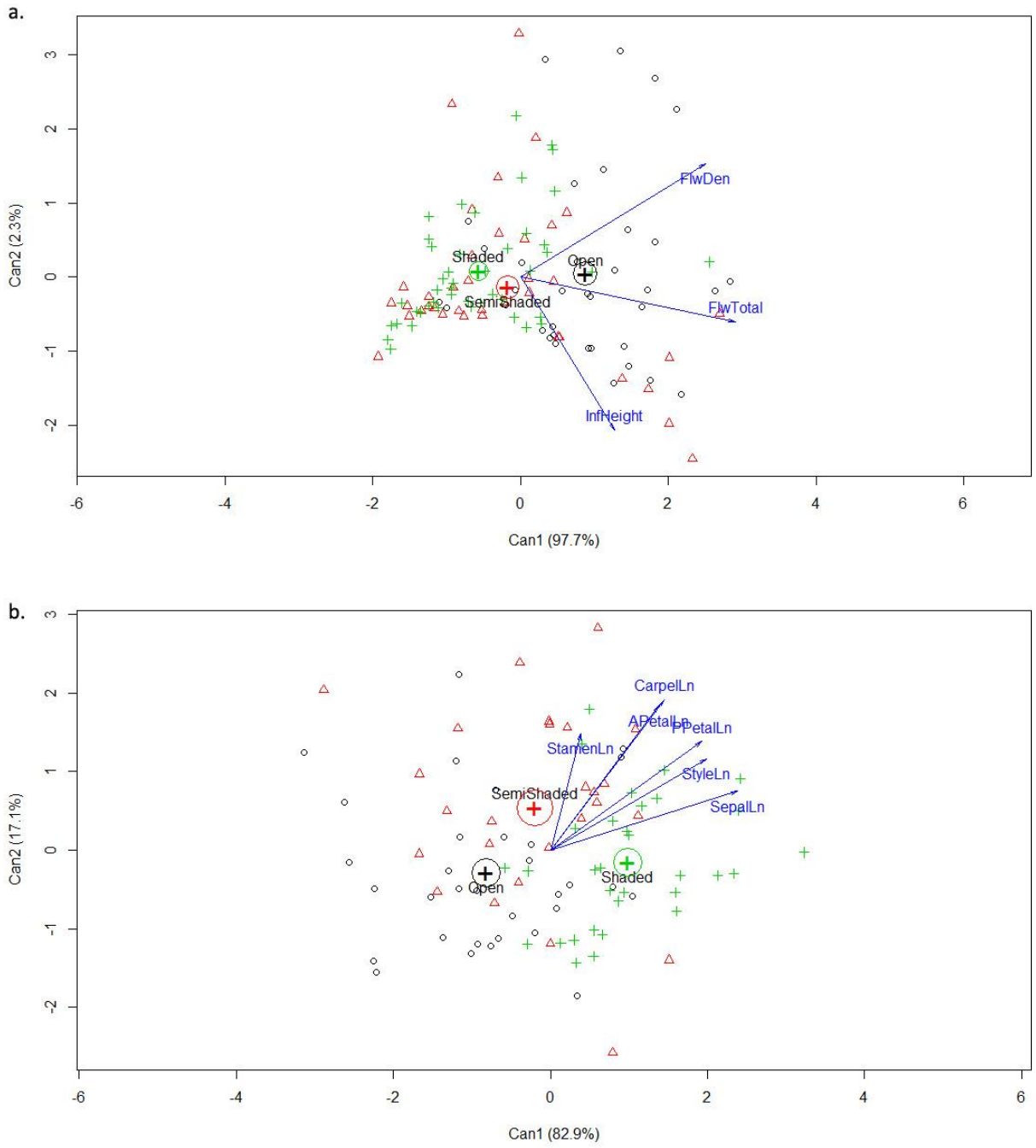

Figure 3.2. Graphical results of canonical discriminant analysis of morphological measurements for *Synthyris bullii* collected from three habitat types: (a) three inflorescence traits, and (b) six flower traits. The trait vectors are plotted on two axes that combine to explain 100% of the variation. The respective habitat types from which flowers were collected are represented these symbols: ○ = open, Δ = semi-shaded, + = shaded.

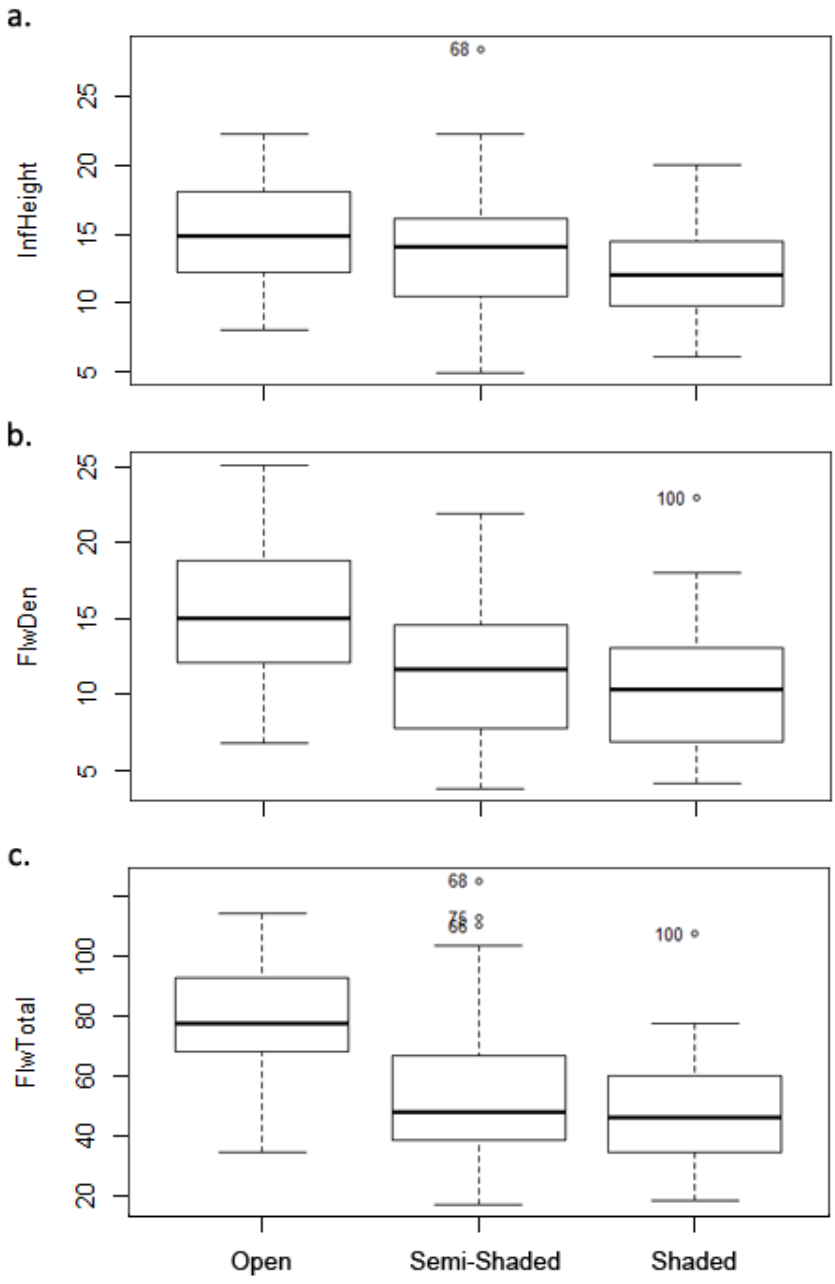

Figure 3.3. Box plots show mean measurements for *Synthyris bullii* plants found in three habitat types: open, semi-shaded, and shaded. The inflorescence traits examined were: (a) total height of the inflorescence, (b) flower density, and (c) total number of flowers per inflorescence.

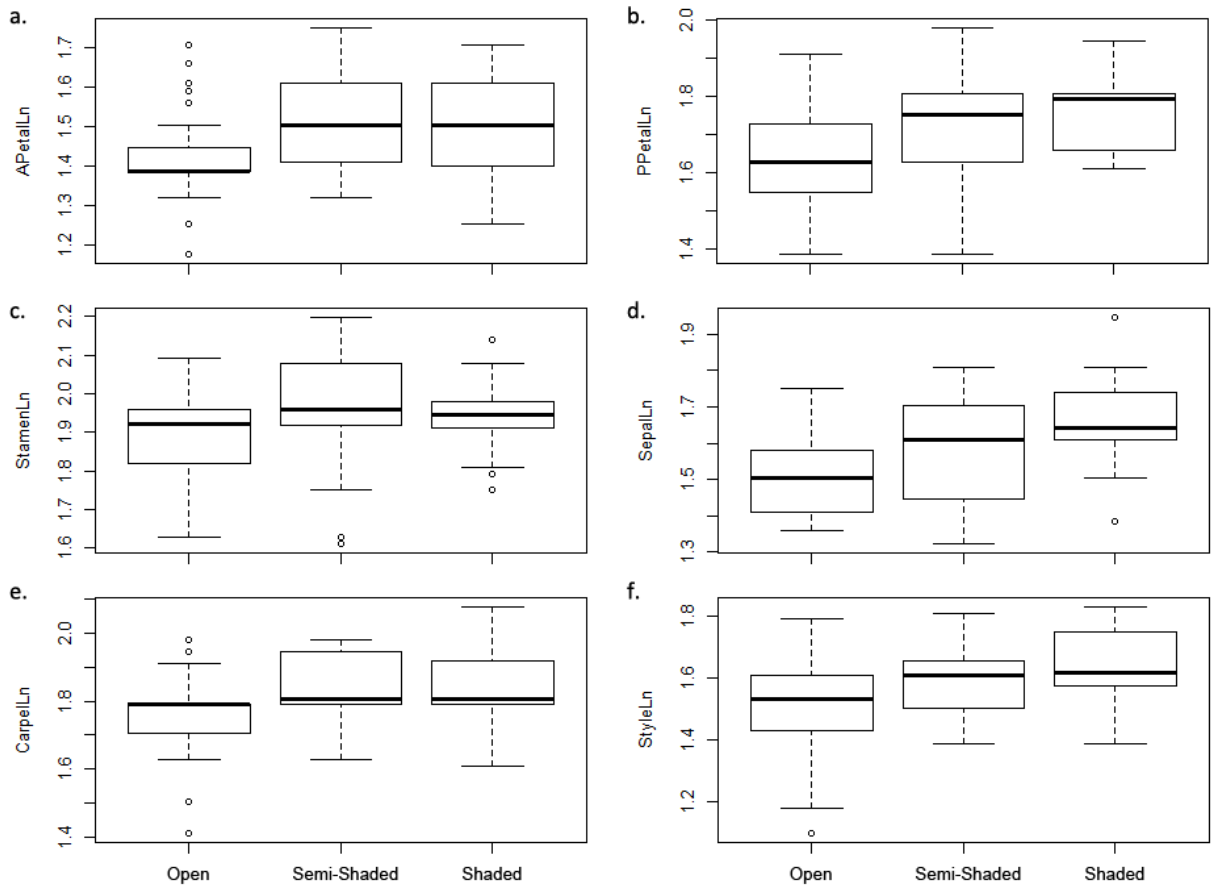

Figure 3.4. Morphometrics of floral traits for *Synthyris bullii* plants found in three habitat types: open, semi-shaded, and shaded. Box plots are shown for lengths of: (a) anterior corolla lobe, (b) posterior corolla lobe, (c) shortest sepal, (d) stamen, (e) carpel, and (f) style. Data were log-transformed prior to analysis.

CHAPTER 4

HABITAT DEGRADATION DISRUPTS PLANT-POLLINATOR INTERACTIONS FOR A RARE, SELF-COMPATIBLE PRAIRIE SPECIES

ABSTRACT

Premise of the Study. Habitat destruction has immediate consequences on biodiversity, whereas the effects of habitat degradation are slower and more subtle. Habitat quality and structure influence reproduction in rare plant species because changes in the local environment can disrupt sensitive plant-pollinator interactions. I used the self-compatible rare species *Synthyris bullii* to examine pollination and reproduction in response to woody encroachment, a type of degradation that occurs in prairies and savannas in the absence of fire. Additionally, I determined if autonomous selfing occurred more frequently in response to reduced pollination in degraded habitats.

Methods. For seven populations, canopy cover and soil characteristics were used to develop three habitat categories that represented different levels of encroachment (e.g., open, semi-shaded, shaded). A pollinator-exclusion experiment was conducted to estimate pollination quantity (i.e., stigma pollen load). Infructescences (n=20) were collected from each population to assess reproductive output (e.g., fruit/seed set) and fitness (i.e., germination).

Key Results. Pollinators contributed 32% to 57% of pollen loads on average. I observed a significant increase in reproductive output associated with the pollinator treatment, even when the relative pollen contribution was small. Further, fruit and seed set were negatively affected by pollinator exclusion regardless of habitat category.

Conclusions. I found evidence that pollination quantity/quality was lower in shaded habitats, which also played a role in lower fruit/seed set and germination compared to other habitats. Autonomous selfing does not occur at a sufficiently high rate, even in shaded habitats, to compensate for pollinator absence. As habitats degrade, reduced pollination quantity/quality and low autonomous selfing rates may contribute to the loss of rare species.

INTRODUCTION

Habitat destruction is recognized as the leading cause of species decline and extinction (Wilcove et al. 1998, Pimm and Raven 2000). However, surviving populations in remnant patches may be threatened over time as a result of habitat degradation, primarily because species adapted to particular habitat conditions may be unable to tolerate changes to the quality and structure of their environment (McKinney and Lockwood 1999, Malcolm et al. 2006, Maskell et al. 2010).

The effects of habitat loss and degradation can be further amplified through the disruption of species interactions, especially mutualisms (Dunn et al. 2009). Rare plant species that depend on animals for pollination are particularly vulnerable as reproduction and recruitment are impeded by the availability and ability of pollinators to visit plants in remnant patches (Kearns and Inouye 1997, Kearns et al. 1998, Wilcock and Neiland 2002, Dunn et al. 2009, Potts et al. 2010). Some plant populations can be sustained in the short term through strategies such as autonomous self-fertilization and clonal reproduction (Honnay and Bossuyt 2005, Van Kleunen et al. 2007, Pauw and Bond 2011). However, the long-term viability of these populations may be compromised by decreased genetic diversity, which is often associated with inbreeding depression and reduced fitness in small, remnant populations (Crnokrak and Roff

1999). Further, reduced genetic diversity limits a population's ability to respond to changing habitat conditions (Honnay and Bossuyt 2005, Honnay and Jacquemyn 2007). This is of particular concern for endangered species as a frequent condition of their rarity is tolerance for only a narrow range of environmental conditions (Rabinowitz 1981).

Much research on habitat loss and plant-pollinator relationships has been devoted to fragmentation, patch size, and other correlates for habitat quality with fewer studies examining drivers of habitat degradation, such as fire and management (Potts et al. 2010). In North America, prairie and savanna remnants suffer from increased habitat degradation due to disruption of historic fire patterns that leads to encroachment by early successional woody species (Leach and Givnish 1996, Briggs et al. 2002, Van Auken 2009). Indeed, research has shown a decrease in species diversity associated with invasion by woody species in grasslands and savannas (Rejmánek and Rosén 1988, Ratajczak et al. 2012). Herbaceous species that characterize these communities experience increased pressure as a result of the ability of woody species to: (1) compete directly for abiotic resources (Wilson 1993, Van Auken and Bush 1997), and (2) alter the abiotic environment (e.g., soil chemistry, canopy structure) in a way that is disadvantageous to herbaceous species (Wilson 1993, Kennedy and Sousa 2006). These changes have direct consequences for rare prairie species as environmental changes and competition for resources may limit the plants' ability for successful reproduction and recruitment.

Shading from woody encroachment also affects access to biotic resources by changing foraging behavior of insect pollinators. As ectothermic organisms, insects are particularly sensitive to microclimatic changes and may avoid shaded areas where temperatures are cooler (Herrera 1995, Culley 2002, Cortes-Palomec and Ballard 2006, Kilkenny and Galloway 2008). Insect avoidance of shading may result in low pollinator visitation for shaded plants, and

subsequently reduced seed set (McKinney and Goodall 2010). This shade-avoidance behavior may be especially true for pollinators that occur in prairies and are presumably adapted to forage in these environments. Due to the absence of pollinators, self-compatibility may also be favored in areas where woody encroachment has deterred insect pollinators. Studies that examine variation in mating systems across species' ranges (i.e., across a gradient of pollinator availability and environmental conditions) often show that selfing rates increase in response to increased physiological stress and pollinator limitation (Busch 2005, Moeller 2006, Moeller et al. 2012).

In the present study, my objective was to examine the effects of woody encroachment, specifically shading, on the reproductive processes of a rare herbaceous species, *Synthyris bullii*, which occurs in open prairie-savanna habitats. Specifically, my study aims to determine: (1) how reproduction and fitness in *Synthyris bullii* are affected by pollination quantity, (2) if pollination quantity, reproductive output (e.g., fruit/seed set), and fitness (i.e., seed germination) are negatively impacted in areas of woody encroachment, and (3) if autonomous selfing is more likely to occur in plants growing in encroached habitats compared to those found in open ones. The results of this study would show if woody encroachment interferes with pollination and reproduction, and could therefore compromise the viability of rare populations.

MATERIAL AND METHODS

Study Species—*Synthyris bullii* (Eaton) A. Heller (Plantaginaceae; Kittentail; synonym *Besseya bullii* (Eaton) Rydb. [*Scrophulariaceae*]) is a rare endemic wildflower of sand-gravel prairies and savannas of the Midwestern United States (Gleason and Cronquist 1991), a region characterized by severe habitat destruction and fragmentation as a result of gravel mining,

agriculture, and urbanization. *Synthyris bullii* is listed as endangered, threatened, or extirpated in all states across its range (Minnesota, Iowa, Wisconsin, Illinois, Indiana, Michigan, and Ohio [extirpated]), and was previously a candidate for federal protection (Fish & Wildlife 1985). Anecdotal accounts in past government assessments speculate that *Synthyris bullii*'s decline may be attributed to declining habitat quality associated with woody encroachment (K. Chi pers. obs.).

In late April and early May, plants in Illinois populations produce one or more inflorescences with 10 to 130 flowers per spike. The flowers of *Synthyris bullii* are hermaphroditic and protogynous (i.e., stigmas are receptive before pollen sheds), and the inflorescence flowers acropetally (i.e., maturing from the base towards the top; McKone et al. 1995). In terms of breeding system, previous studies have determined *S. bullii* to be at least self-compatible and pollinated by bees (McKone et al. 1995, Cholewa unpub. data).

Study Sites and Habitat Classification—Seven populations located in northwestern Illinois were selected for the study (Table 4.1). Based on canopy cover, populations were assigned to one of three habitat categories: open, shaded and semi-shaded (Table 4.1). For canopy cover, the "openness" of sites was estimated using canopy cover, or the amount of sky that was visible when accounting for obstructions from nearby woody vegetation. To estimate canopy cover, ten collection points were set up at a regular interval across the span of each population. At each collection point, the canopy was photographed using a 0.7-meter-tall tripod and Sigma SD14 SLR camera fitted with a fisheye lens. Photographs were imported into Canopy Analysis Software 2.1 to estimate the percentage of sky that was visible from the perspective of plants in the population (Appendix E).

Pollination Quantity—A preliminary study was conducted to establish that pollinators were visiting *S. bullii* at study sites. Approximately 24 hours of observations were conducted on clear, sunny days during the morning and afternoon. Each patch of three to five plants was watched for 15-minute intervals for pollinator activity. Only pollinators that made contact with stigmas or anthers were noted. We determined that the primary visitors of *S. bullii* inflorescences were halictid bees and bumblebees. Pollinators were active during early afternoon hours. These observations were consistent with earlier studies conducted on this species (McKone et al. 1995).

As a proxy for pollinator visitation, I used stigma pollen load as it has been previously shown that there is a relationship between pollinator activity and pollen load size (Engel and Irwin 2003). Measuring pollination quantity via stigma pollen load was chosen as my tool for estimating pollinator activity because pollinator visitation at my sites was infrequent and difficult to accurately capture using only field observations. Further, an exclusion experiment was used to determine pollination quality (self-pollen vs. cross-pollen) and rates of autonomous selfing. *S. bullii*'s breeding system has been well-studied and it has been found that plants do not differ in fruit set when dusted with self- vs. outcross-pollen (McKone et al. 1995, Cholewa unpub. data). Thus, I chose not to repeat experiments on this plant's breeding system. Instead, I assumed that stigma pollen loads for excluded plants must originate exclusively from the same plant. Moreover, I assumed that any fruits or seeds that develop in the exclusion treatment are the products of self-pollen and reflects the reproductive output that results under natural pollinator limitation.

Prior to flowering in each population, ten plants were haphazardly selected for pollinator-exclusion and another ten plants were selected for a control treatment (i.e., accessible to pollinators). Plants were selected by tossing a ball blindly within the population and tagging the

nearest plant. To avoid potential confounding resource effects involved with producing multiple inflorescences, only those plants producing a single inflorescence were chosen.

Individuals in the pollinator-exclusion treatment were covered in mesh cages wrapped in a thin layer of bridal veil that prevented contact from insects or other plants, and only minimally interferes with light exposure (Kearns and Inouye 1993). Plants in the exclusion treatment were checked weekly to ensure that no part of the inflorescence contacted edges of the cage. Control treatment plants were marked with a tag but no other manipulation occurred.

From each plant in both treatments, three stigmas were randomly collected to determine pollen load size per inflorescence. Stigmas were excised once female-phase flowers had fully transitioned to male-phase flowers and were no longer receptive, and stored in formalin-acetic acid-alchol (FAA) at room temperature. To assess pollen load size, stigmas were stained with a 20% concentration aniline blue dye for one minute. After being fixed on microscope slides with glycerin, stigmas were then examined under a transmitted light microscope using AxioVision 4.7 software and all visible pollen grains were counted.

Reproductive Output and Fitness—After fruit had set, infructescences from the two treatments were collected from each population. The total number of fruit and unfertilized flowers were counted for each infructescence. Fruit set was calculated as the proportion of fruit that had successfully formed out of the total number of flowers in an inflorescence. For each infructescence, five fruit were randomly selected to determine a mean seed set. Seed set was calculated as the proportion of seeds that had formed out of the total number of ovules determined for *S. bullii* (n=52, Appendix D).

Seed viability for the two treatments was determined through a germination study in a growth chamber. From each infructescence, 80 seeds were collected from different fruits, mixed,

and separated into 4 petri dishes lined with moistened filter paper. For the cold stratification process, petri dishes were individually wrapped in aluminum foil and stored at 3°C for three months. Afterward, petri dishes were unwrapped and placed in a growth chamber that simulated spring conditions (20°C, 14-hour photoperiod). Seeds were monitored daily for four weeks to check for signs of germination; specifically, emergence of roots and cotyledons.

Statistical Analysis—Prior to analysis, all data was tested for normality by examining Q-Q Plots and calculating *W*-values using the Shapiro-Wilk statistic with PROC UNIVARIATE in SAS software (SAS Institute 2011). Data was considered normal when $W \geq 0.90$ and $p \geq 0.50$. To achieve a normal distribution, data for the pollinator exclusion experiment was transformed in the following ways: pollen data was log-transformed, and seed set and germination were transformed using $arcsin(\sqrt{x})$.

The effects of pollination quantity were analyzed by modeling the relationship between stigma pollen load and reproduction and fitness using PROC GLM. A factorial analysis in PROC GLM was used to examine main effects and interaction effects of treatment and habitat on reproduction, followed by a Tukey's *post hoc* test. All statistical analyses were conducted using SAS (SAS Institute 2011)

RESULTS

Effects of Pollination Quantity on Reproduction— When examining stigma pollen load, the number of pollen grains per load ranged from 1 to 192. Fruit set and seed set showed a positive trend with increasing stigma pollen load size, while germination appeared to have no relationship with pollen quantity (Fig. 4.1). Fruit set showed a significant positive relationship with pollen load (R^2=0.659, p<0.001). The additional pollen in the control treatment led to

increases in fruit set for all study populations, though the magnitude of increase varied among populations (Fig. 4.1a). For example, compared to the exclusion treatment, stigma pollen load was higher in the control by 33% in FUL and 34% in NAT, and this led to an increase in fruit set of 82% and 41%, respectively.

Similarly, seed set was found to increase with pollen load (R^2=0.360, p=0.023). As with fruit set, the magnitude of increase was not consistent among populations (Fig. 4.1b). Some populations showed a considerable increase in seed set when comparing the two treatments, such as FUL where a 33% increase in pollen load in the control vs. exclusion led to a 49% increase in seed set. By comparison, a 49% increase in pollen load in the control treatment vs. exclusion for LMR corresponded with only a 9% increase in seed set.

There was no relationship between germination and pollen load (R^2=0.010, p=0.733). The addition of pollen in the control compared to exclusion treatment did not result in an increase in seed germination (Fig. 4.1c).

Pollination Quality and Habitat Categories—Treatment was found to have a significant effect on pollen loads (df=1, F=27.41, p<0.001), but neither habitat (df=2, F=0.90, p=0.414) nor the interaction (df=2, F=0.90, p=0.417) were found to be significant. When pollinators were excluded from plants, there was a strong significant decrease in stigma pollen load for all habitat types. Pollinator exclusion reduced stigma pollen load size by approximately 57% for open habitats, 49% for semi-shaded habitats, and 32% for shaded habitats. Although stigma pollen load did not differ significantly among habitat types, there was on average 30% fewer pollen grains on stigmas collected from control plants in shaded habitats compared to open and semi-shaded ones (Fig. 4.2a).

Habitat Effects on Reproductive Output and Fitness—Proportion of fruit set for plants ranged from 0.07 to 1.00. Significant differences were found for both main effects: treatment (df=1, F=207.63, p<0.001) and habitat (df=2, F=14.62, p<0.001); there was no significant interaction (df=2, F=1.10, p=0.336). In terms of treatment effect, pollinator-excluded plants produced significantly lower fruit set compared to control plants. Fruit set differed significantly among habitat types, with shaded habitat having lower fruit set than open and semi-shaded habitats (Fig. 4.2b).

When measuring seed production, it was found that the proportion of seed set ranged from 0 to 0.42. Both main effects were found to be significant, but there was no interaction between main effects (df=2, F=0.97, p=0.383). Seed set was negatively affected by pollinator exclusion, resulting in lower seed set in pollinator-excluded plants than control plants (df=1, F=22.20, p<0.001). Seed set also differed significantly among habitat types (df=2, F=8.41, p<0.001). Shaded habitats showed significantly lower seed set than semi-shaded habitats (Fig. 4.2c).

Seed germination for *S. bullii* was found to be relatively high, with a proportion of 0.55 to 1.00 seeds germinating successfully. Significant differences were found among habitats (df=2, F=4.90, p=0.011), but not for treatment (df=1, F=0.01, p=0.913) or the interaction (df=2, F=0.72, p=0.492). Specifically, germination in semi-shaded habitats differed significantly from shaded habitats, while open habitats did not differ from either semi-shaded or shaded habitats (Fig. 4.2d).

DISCUSSION

Breeding System and Pollination Quantity—Fewer pollen grains were found on the stigmas of pollinator-excluded plants compared to plants with pollinator access (Fig. 4.1a). In the case of self-compatible, dichogamous species, pollinators can improve reproductive output through: (1) dispersal of pollen between plants, and/or (2) facilitation of geitonogamous self-fertilization when moving pollen between flowers on the same plant (Lloyd and Schoen 1999, Eckert 2000). The size of pollen loads is associated with pollinator access, which in turn has an important role in reproductive output (Brown and Kephart 1999). For example, plants without pollinator access generally had pollen loads ranging from 10 to 25 grains and a decreased fruit and seed set compared to control plants (i.e., open pollinated), which had pollen loads ranging from 25 to 45 grains and increased fruit and seed set.

In my study, the positive relationship between stigma pollen load and fruit/seed set suggests that there is a threshold amount of pollen that is required to trigger successful fruit/seed formation, and that this would likely require pollinators to deliver large pollen loads to flowers. However, when examining the relationship between stigma pollen load and fruit set (Fig. 1a), some populations in the control treatment (e.g., PAO, FUL) produced much higher fruit set even though pollen loads were small and comparable in quantity to pollen loads from the exclusion treatment. For example, when comparing the control and exclusion treatments for FUL, I found that a mean increase of 8 pollen grains on stigmas in the control treatment saw a marked increase of 82% in fruit set. Considering I collected samples with as many as 200 pollen grains per stigma, it seems surprising to observe that a small difference in pollen grains yielded such a substantial increase in fruit set. This disproportionately powerful effect points to other factors that may be at play.

While reproductive output showed a positive relationship with stigma pollen load in *S. bullii*, pollen quantity is unlikely to be the sole explanation for observed patterns in all populations. In a few populations, marginal increases in pollen load between control and excluded treatments resulted in disproportionately large increases in fruit set. Pollen quality, specifically outcross pollen, may have also contributed to improved reproductive output for my control plants (i.e., open pollinated). Although *S. bullii* has been demonstrated to be self-compatible (McKone et al. 1995, Cholewa unpub data), past studies similarly show that selfed offspring have been found to be less fit compared to outcrossed offspring in other self-compatible species (de Jong et al. 1993, Husband and Schmeske 1996). Consequently, these results show that autonomous selfing cannot support reproduction alone, and that there is evidence that pollen quality (i.e., outcross pollen) plays an important role in reproductive outcomes, even for self-compatible species.

Interestingly, while fruit and seed set are reduced by the exclusion of pollinators, seed germination was not affected. All seeds showed equal germinability, regardless of pollination quantity/quality. Because successful fertilization by self-pollen can still result in viable seeds, it appears that reduced pollen quantity interferes only with fruit and seed formation. However, other studies have demonstrated that germination is artificially high under ideal laboratory conditions, and that fitness consequences only manifest in a natural field context (Dudash 1990, Ramsey & Vaughton 1998, Morgan 2001). Indeed, a study of *S. bullii* under different simulated environments resulted in variable seed germination (Curtis et al. 2013). In addition, while my study only examined seed viability, negative fitness consequences from selfing may still manifest at later life stages in terms of offspring survival and reproduction (Dudash 1990,

Johnston 1992). While I found no germination differences in response to pollen quantity/quality, there may nevertheless be fitness consequences for traits that I failed to capture in this study.

Habitat Effects on Reproduction—Habitat conditions associated with woody encroachment may cause physiological stress or limit access to important resources (i.e., sunlight) (Van Auken and Bush 1997), which my study shows negatively impacts reproduction. Because plants require light for both photosynthetic activity and as an environmental cue, shading can considerably alter the growth, development, and biomass allocation of herbaceous species (Slade & Hutchings 1987, Stuefer & Huber 1998). Differences in light availability and soil nutrient composition among habitat types (Table 1) may play an important role in terms of the resources available for reproduction functions.

However, while these abiotic factors associated with encroachment may have negative consequences for plant reproduction, my study shows that a compelling explanation for decreased reproductive output in encroached areas involves reductions in pollen quantity and quality. Using stigma pollen load size as an indicator of pollinator activity, I determined that pollination quantity and quality is similar in open, semi-shaded, and shaded habitats (Fig. 3a). However, while the difference among habitat categories was found to be statistically non-significant, within the open treatment I still observed pollen loads that were 30% smaller on average in shaded habitats relative to the other two habitats, suggesting that pollination quantity is lower in shaded areas. My results support the idea that encroachment has negative impacts on pollinator visitation for *S. bullii*. As woody encroachment changes habitat quality and structure, the optimal pollinators associated with these plant species may be lost (Potts et al. 2010)—or may even change their foraging behavior in response to the microclimatic conditions associated with increased shading (Kilkenny and Galloway 2008)—which has significant negative

consequences for plant-pollinator relationships and the reproductive success of threatened species.

In addition, when comparing the stigma pollen load of control and pollen-excluded plants, it is apparent that self-pollen can make up as much as 68% of total pollen loads of natural plants in shaded habitats, suggesting that pollinators in encroached areas are making somewhat limited contributions toward pollen deposition. The high proportion of self-pollen in the pollen loads of encroached areas (i.e., lower pollen quality) is of particular concern because self-pollen can contribute to reduced fitness even in self-compatible species (de Jong et al. 1993, Husband and Schmeske 1996). Indeed, when comparing the control treatment for all habitats, fruit and seed set for shaded populations were significantly lower than the other two habitat categories (Fig. 3a,b). Taken together, this shows that the reduced reproductive output observed in encroached areas may be caused by a combination of reduced pollinator activity and increased fertilization by self-pollen.

CONCLUSIONS

While other studies have established patterns of species decline following woody encroachment (Rejmánek and Rosén 1988, Ratajczak et al. 2012), the research presented here suggests that habitat changes driven by encroachment could negatively impact rare plant species through deterrence of insect pollinators and interference with reproduction. Over time populations with inadequate pollinator visitation may begin to favor selfing (Moeller et al. 2012), yet my study shows that plants in encroached habitats are visited less frequently by pollinators, and these populations are not responding by increased autonomous selfing or successful fertilization by self-pollen. Pollinators contribute only a portion of pollen to stigma pollen loads,

and even in cases where this contribution appears marginal, the quantity and quality of cross-pollen has a significant positive impact on reproductive success. Because autonomous selfing failed to occur at sufficient levels to allow populations to escape the need for pollinators in encroached areas, I conclude that habitat degradation disrupts plant-pollinator interactions in a way that compromises the viability of populations of rare plant species. These results indicate a strong need to manage areas of prairie and savanna where aggressive invasion by woody vegetation alters the local ecology and interferes with sensitive plant-pollinator relationships.

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TABLES AND FIGURES

Table 4.1. Study populations of *Synthyris bullii* were assigned to one of three habitat categories. The three habitat categories were characterized using canopy cover (means ± SEM).

Site Name	Habitat Type	Mean Visible Sky (%) ± SEM
LMF	Open	92.1 ± 0.04
LMR	Open	100.0 ± 0.00
NAK	Semi-Shaded	56.9 ± 0.02
NAT	Semi-Shaded	80.2 ± 0.01
FUL	Shaded	37.9 ± 0.04
PAO	Shaded	9.90 + 0.01
PAM	Shaded	14.0 ± 0.00

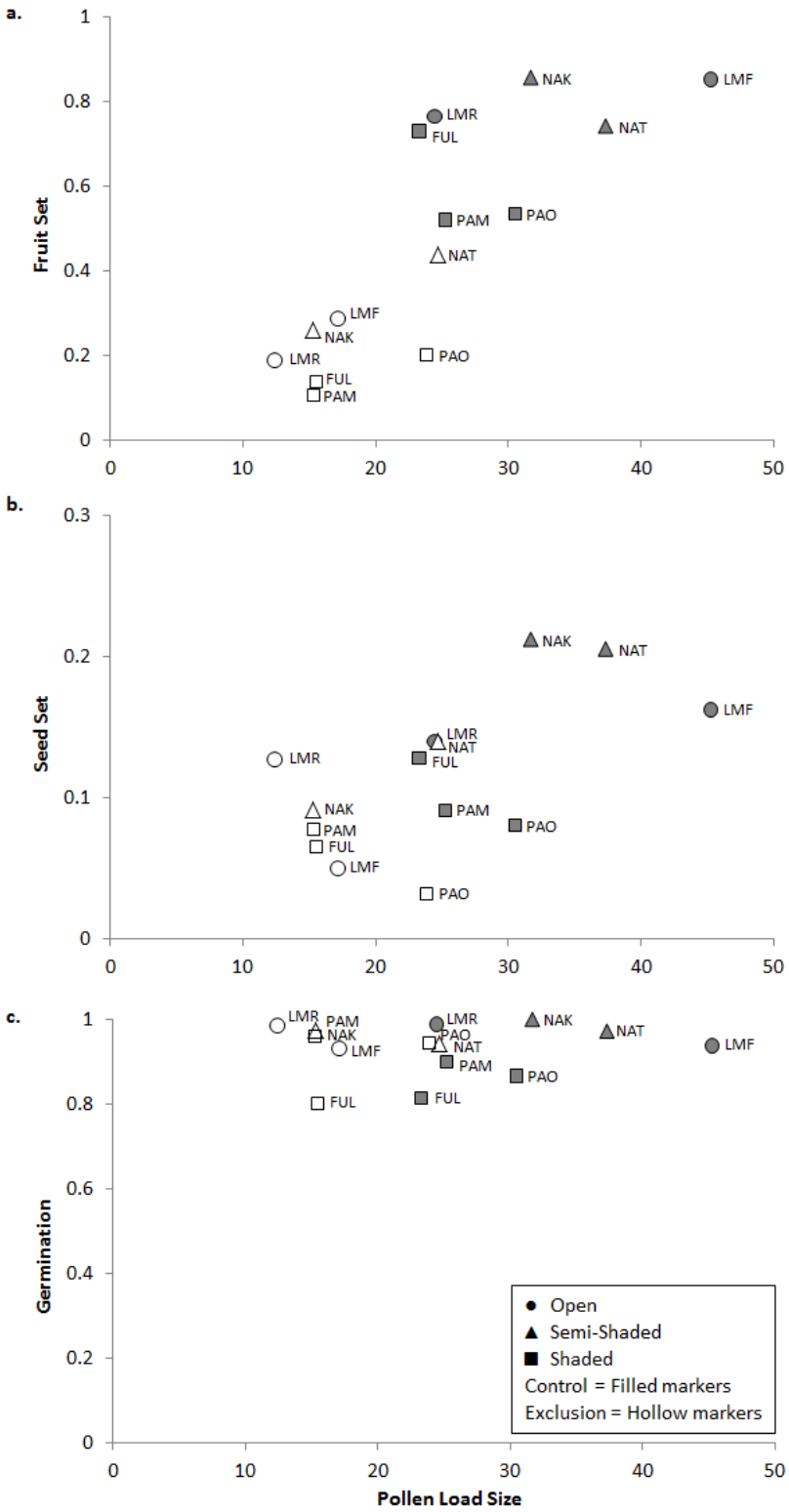


Figure 4.1. Relationship between pollen load (i.e., mean number of pollen grains on stigma) and: (a) fruit set, (b) seed set, and (c) seed germination for *Synthyris bullii* populations used in the study.

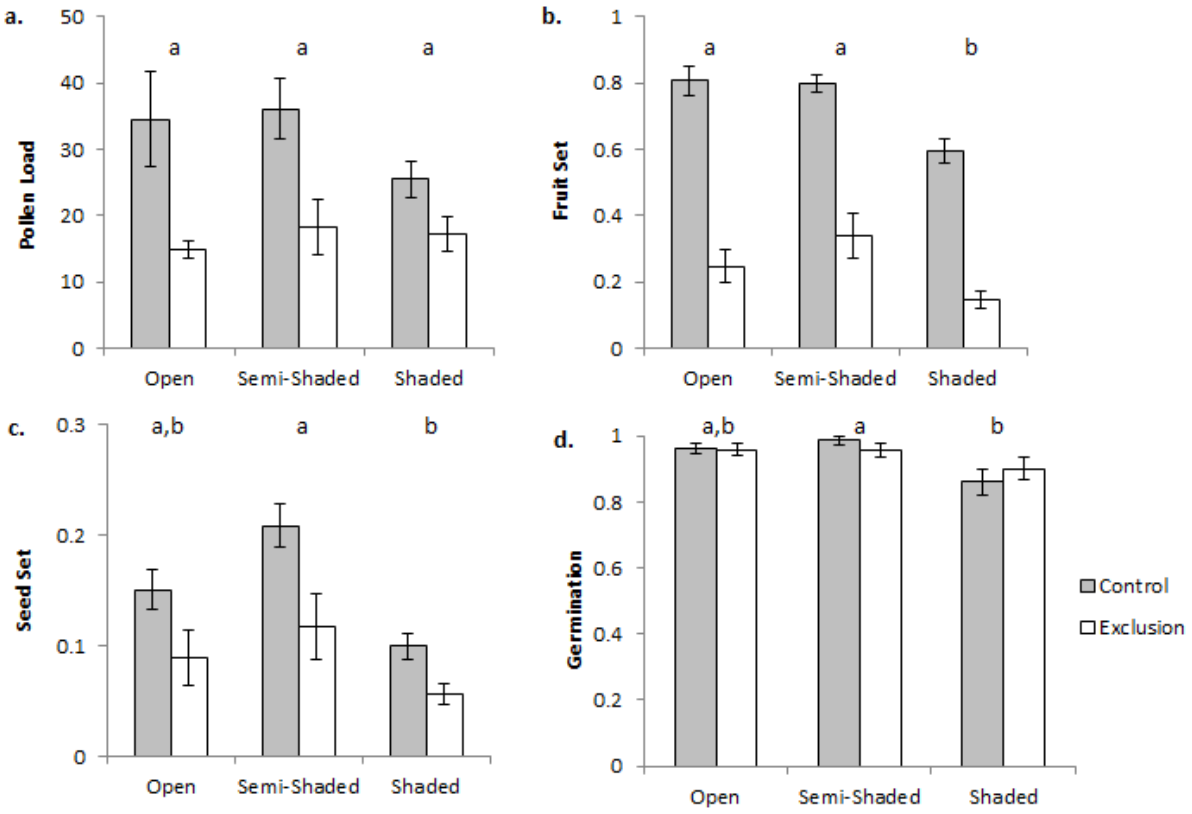


Figure 4.2. Reproduction for *Synthyris bullii* in control and pollinator-excluded treatments for three habitats, showing means (± SEM) for (a) number of pollen grains on stigmas, (b) proportion of fruit set, (c) proportion of seed set, and (d) seed germination. Pollinator exclusion treatment was found to significantly affect all reproductive variables except for germination. Alphabetical letters denote significant differences ($p \leq 0.05$) among habitat types.

CHAPTER 5

CONCLUSION

A goal of this thesis was to provide an examination of the demographic, environmental, and management factors that can influence conservation efforts for rare plants. In North America, habitat destruction has contributed to a major loss of biodiversity, and subsequent habitat fragmentation and degradation continues to create challenges for rare populations, especially in terms of their reproductive ecology.

The results from Chapter 1 show that there are reproductive consequences resulting from demographic factors. While fruit set shared a positive relationship with floral display quality, inflorescence size and seed set were negatively impacted by small population size. The positive relationships between population size and inflorescence floral display and seed set may be indicative of improved resource quality at these sites--that is, these particular areas were able to support more robust, reproductively successful populations compared to smaller, sparser populations with reduced inflorescence size and seed set.

Moreover, the combination of results from Chapters 2 and 3 confirmed that flower morphology and reproductive success were associated with specific habitat types. The results of Chapter 2 showed that plants may experience a tradeoff between inflorescence and flower size, resulting in smaller inflorescences with larger flowers in shaded habitats. Taking Chapters 1 and 2 together, I might assume that the smaller inflorescence size associated with shaded habitats should have a negative effect on fruit set. Indeed, when comparing reproductive output among different habitat types in Chapter 3, I did find reduced fruit and seed set in shaded habitats. Increased fruit and seed set for plants growing at open sites may also be a result of improved

pollinator visitation at these sites, as my experimental manipulation suggested that larger pollen loads may be found on flowers in open habitats compared to shaded ones.

From a management perspective, these results have a few implications. First, population size and habitat openness have a positive influence on reproductive success for this rare prairie-savanna species. However, the ability to increase population size and reproductive output may be limited by habitat quality and the ability of sites to support reproduction and recruitment. Major conservation strategies for plants like my study species thus depend on efforts to manage habitat quality, such as mechanical removal of woody vegetation and use of fire to prevent establishment of new woody growth. Results from my four-year study in Illinois confirm that reproduction responds positively to the presence of management and increased habitat openness.

APPENDIX A

Populations of *Synthyris bullii* in Illinois were surveyed during 2008 and 2009 to assess: number of flower plants (Flw), percentage of flowering plants (Flw %), total number of plants (Tot Pop), total area of population (Area), density of flowering plants (Flw Dens), and density of all plants (Total Dens). The table also shows code names used for populations throughout the thesis.

				2008					
Population	**Code**	**State**	**Habitat**	**Flw**	**Flw (%)**	**Total Pop**	**Area (m^2)**	**Flw Dens**	**Total Dens**
LoMoF105	LMF	IL	Open						
LoMoRail	LMR	IL	Open	304	39.9	762	135.06	2.2509	5.6402
NachusaKit	NAK	IL	Semi	500	50.0	1000	1212.0	0.4125	0.8251
NachusaTell	NAT	IL	Semi	85	21.9	389	57.008	1.4910	6.8236
HarlemHills	HAH	IL	Open	93	44.5	209	29.20	3.1849	7.1575
Fulton	FUL	IL	Shaded	106	22.5	472	1180.6	0.0898	0.3998
PalisadesMush	PAM	IL	Shaded	239	37.6	635	1222.2	0.1955	0.5195
PalisadesOzz	PAZ	IL	Shaded	20	50.0	40	53.057	0.3770	0.7539
PalisadesOak	PAO	IL	Shaded	253	56.1	451	287.48	0.8801	1.5688
KinnCreek	KCK	IL	Open						
ShirWinter	SHW	IL	Open						
ShirHolmes	SHH	IL	Semi						
BigRiver	BGR	IL	Shaded						
DaysCem	DYV	IL	Open						

				2009					
Population	**Code**	**State**	**Habitat**	**Flw**	**Flw (%)**	**Total Pop**	**Area (m^2)**	**Flw Dens**	**Total Dens**
LoMoF105	LMF	IL	Open	511	26.99	1893	215.82	2.3677	8.7713
LoMoRail	LMR	IL	Open	398	28.29	1407	135.06	2.9469	10.418
NachusaKit	NAK	IL	Semi	271	18.25	1485	1212	0.2236	1.2252
NachusaTell	NAT	IL	Semi	79	82.29	96	57.008	1.3858	1.684
HarlemHills	HAH	IL	Open	79	18.33	431	29.2	2.7055	14.76
Fulton	FUL	IL	Shaded	47	4.84	972	1180.6	0.0398	0.8233
PalisadesMush	PAM	IL	Shaded	175	50.00	350	1222.2	0.1432	0.2864
PalisadesOzz	PAZ	IL	Shaded	8	11.76	68	53.057	0.1508	1.2816
PalisadesOak	PAO	IL	Shaded	92	25.77	357	287.48	0.3200	1.2418
KinnCreek	KCK	IL	Open	31	18.24	170			
ShirWinter	SHW	IL	Open	13	68.42	19			
ShirHolmes	SHH	IL	Semi	5	50.00	10			
BigRiver	BGR	IL	Shaded	11	36.67	30			
DaysCem	DYV	IL	Open	11	7.38	149			

APPENDIX B

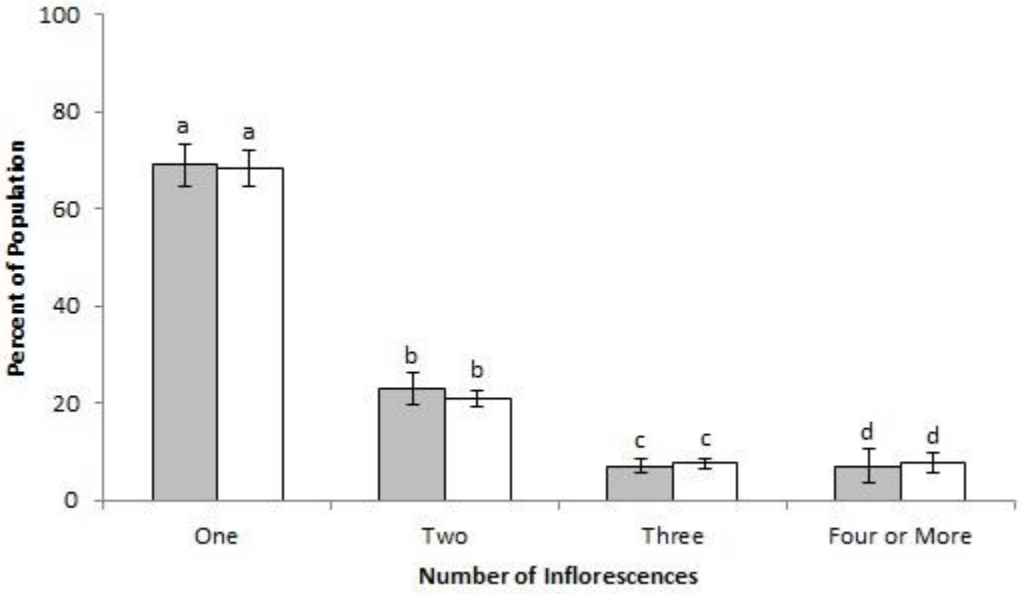
During 2010 and 2011, populations of *Synthyris bulli* were surveyed across the species' range. Data in the following table show: number of flower plants (Flw), percentage of flowering plants (Flw %), total number of plants (Tot Pop), total area of population (Area), density of flowering plants (Flw Dens), and density of all plants (Total Dens). The table also shows code names used for populations throughout the thesis.

				2010					
SiteName	**Code**	**State**	**Habitat**	**Flw**	**Flw (%)**	**Total Pop**	**Area (m^2)**	**Flw Dens**	**Total Dens**
LoMoF105	LMF	IL	Open	158	20.33	777	215.82	0.7321	3.6003
LoMoRail	LMR	IL	Open	220	36.6	601	135.06	1.6289	4.4499
NachusaKit	NAK	IL	Semi	708	26.67	2654	1212	0.5841	2.1897
NachusaTell	NAT	IL	Semi	66	19.7	335	57.008	1.1577	5.8764
HarlemHills	HAH	IL	Open	78	39.79	196	29.2	2.6712	6.7123
Fulton	FUL	IL	Shaded	23	1.52	1489	1180.6	0.0195	1.2612
PalisadesMush	PAM	IL	Shaded	42	26.08	161	1222.2	0.0344	0.1317
PalisadesOzz	PAZ	IL	Shaded	0	0	42	53.057	0	0.7916
PalisadesOak	PAO	IL	Shaded	18	9.42	191	287.48	0.0626	0.6644
ACWoods	ACW	WI	Open	22	66.67	33	12.284	1.7909	2.6864
Tichigan	TCH	WI	Shaded	1	1.79	56	245.77	0.0041	0.2278
Lakewood	LKW	WI	Semi	25	20	125	26.569	0.9409	4.7047
EagleCentre	EGC	WI	Shaded	287	24.28	1182	5421.7	0.0529	0.218
LuluLake	LUL	WI	Semi	6	40	15	10.11	0.5935	1.4837
MuraltBluff	MUB	WI	Open	71	20.29	350	51.232	1.3859	6.8317
AlbanyRR	ARR	WI	Shaded	2	33.33	6	5.231	0.3823	1.1470
Alexander	ALX	WI	Shaded	75	54.35	138	254.43	0.2948	0.5424
FosterCemetery	FOS	WI	Shaded	18	40	45	39.372	0.4572	1.1429
StandCedar	STC	WI	Semi	18	11.25	160	158.18	0.1138	1.0115
BuffaloSkull	BFS	WI	Semi	6	23.08	26	29.583	0.2028	0.8789
CedarBottoms	CDB	IA	Open	371	15.98	2322	3638.9	0.102	0.6381
KleinCemetery	KLC	IA	Open	43	70.49	61	20	2.15	3.05
GreenIsland	GRI	IA	Shaded	17	28.33	60	5.19	3.2755	11.6054
AndersonPrairie	ADS	IA	Open	137	56.61	242	4424.2	0.031	0.0547
BellePrairie	BLP	MN	Shaded	0	0	43	4.903	0	8.7701
RiverTerrace	RTR	MN	Open	113	20.25	558	1016.9	0.1111	0.5487
CarpenterNC	CPT	MN	Shaded	25	18.38	136	345.75	0.0723	0.3933
WeaCreek	WCR	IN	Semi	13	33.33	39	11.777	1.1038	3.3115
FawnRiver	FWR	IN	Shaded	0	0	17	33.08	0	0.5139
ThornappleBluff	THN	MI	Shaded	11	40.74	27	63.386	0.1735	0.426
WolfLakeRoad	WLF	MI	Shaded	0	0	45	76.293	0	0.5898

				2011					
SiteName	**Code**	**State**	**Habitat**	**Flw**	**Flw (%)**	**Total Pop**	**Area (m^2)**	**Flw Dens**	**Total Dens**
LoMoF105	LMF	IL	Open	127	20.48	620	215.82	0.5885	2.8728
LoMoRail	LMR	IL	Open	186	36.19	514	135.06	1.3772	3.8057
NachusaKit	NAK	IL	Semi	70	8.16	858	1212	0.0578	0.7079
NachusaTell	NAT	IL	Semi	69	51.11	135	57.008	1.2104	2.3681
HarlemHills	HAH	IL	Open	54	37.76	143	29.2	1.8493	4.8973
Fulton	FUL	IL	Shaded	35	6.55	534	1180.6	0.0296	0.4523
PalisadesMush	PAM	IL	Shaded	55	39.29	140	1222.2	0.045	0.1145
PalisadesOzz	PAZ	IL	Shaded	13	30.95	42	53.057	0.245	0.7916
PalisadesOak	PAO	IL	Shaded	89	50.57	176	287.48	0.3096	0.6122
ACWoods	ACW	WI	Open	8	25.81	31	12.284	0.6513	2.5236
Tichigan	TCH	WI	Shaded	12	17.14	70	245.77	0.0488	0.2848
Lakewood	LKW	WI	Semi	40	25.97	154	26.569	1.5055	5.7962
EagleCentre	EGC	WI	Shaded	87	26.04	334	5421.7	0.016	0.0616
LuluLake	LUL	WI	Semi				10.11	0	0
MuraltBluff	MUB	WI	Open	74	33.33	222	51.232	1.4444	4.3332
AlbanyRR	ARR	WI	Shaded				5.231	0	0
Alexander	ALX	WI	Shaded	95	50.27	189	254.43	0.3734	0.7428
FosterCemetery	FOS	WI	Shaded	28	50.91	55	39.372	0.7112	1.3969
StandCedar	STC	WI	Semi	111	35.13	316	158.18	0.7017	1.9977
BuffaloSkull	BFS	WI	Semi				29.583	0	0
CedarBottoms	CDB	IA	Open	3114	57.36	5429	3638.9	0.8557	1.4919
KleinCemetery	KLC	IA	Open				20	0	0
GreenIsland	GRI	IA	Shaded	5	14.71	34	5.19	0.9634	6.5511
AndersonPrairie	ADS	IA	Open	1313	53.92	2435	4424.2	0.2968	0.5504
BellePrairie	BLP	MN	Shaded				4.903	0	0
RiverTerrace	RTR	MN	Open	725	41.31	1755	1016.9	0.7129	1.7258
CarpenterNC	CPT	MN	Shaded	53	36.3	146	345.75	0.1533	0.4223
WeaCreek	WCR	IN	Semi	4	12.9	31	11.777	0.3396	2.6322
FawnRiver	FWR	IN	Shaded	0	0	8	33.08	0	0.2418
ThornappleBluff	THN	MI	Shaded	11	84.62	13	63.386	0.1735	0.2051
WolfLakeRoad	WLF	MI	Shaded	0	0	12	76.293	0	0.1573

APPENDIX C

The mean percentage of individuals in *Synthyris bullii* populations that produced one or more inflorescences. Dark and light bars represent data collected during 2010 and 2011, respectively. On average, 67% of plants in a population produce one inflorescence whereas very few plants produce three or more inflorescences.



APPENDIX D

Flowers randomly sampled from three sites were dissected to determine mean ovule number for *Synthyris bullii*. Raw data for ovule counts are shown below. Mean ovule number was not found to differ significantly among sites (ANOVA: F_2=0.686, P=0.511).

Population	Sample	Ovule Number
HAH	1	65
	2	45
	3	69
	4	60
	5	57
	6	56
	7	34
LMF	1	44
	2	31
	3	67
	4	44
	5	63
	6	48
LMR	1	54
	2	41
	3	45
	4	58
	5	55
	6	46
	7	32
	8	49
	9	43
	10	55
	11	56
	12	43
	13	45
	14	58
	15	66
	16	55
	17	49
	18	58
	19	55
	20	48

APPENDIX E

Site "openness" was estimated for seven populations using canopy cover (i.e., the amount of sky that was visible after taking into account obstructions from nearby woody vegetation [= Proportion Visible Sky]). To estimate canopy cover, collection points were set up at a regular interval across the span of each population. At each collection point, the canopy was photographed using a 0.7-meter-tall tripod and Sigma SD14 SLR camera fitted with a fisheye lens. Photographs were imported into Canopy Analysis Software 2.1 to estimate the proportion of visible sky above each population. No data were collected for LMR as there was no canopy cover whatsoever at any point at that site.

Population	Sample	Proportion Visible Sky
FUL	1	0.42032254
	2	0.337790706
NAK	1	0.353428418
	2	0.491197942
	3	0.602191032
	4	0.53265997
	5	0.595500217
	6	0.575683801
	7	0.57704458
	8	0.613505098
	9	0.644831184
	10	0.654118058
	11	0.592101643
	12	0.593198001
NAT	1	0.808076013
	2	0.833750668
	3	0.925661857
	4	0.934876196
	5	0.944719572
	6	0.94385913
	7	0.924827192
	8	0.908212813
	9	0.857711038
	10	0.923303768
LMF	1	0.8990813
	2	0.776273004
	3	0.988352732
	5	0.594850723
	6	0.990750613
	7	0.999202474
	8	0.960152017

	9	0.996209764
	10	0.982396028
	11	0.973096655
	12	0.941103303
	13	0.832269672
PAM	1	0.104005021
	2	0.132102981
	3	0.162082329
	4	0.122270931
	5	0.16031827
	6	0.144797332
	7	0.128258916
	8	0.158255182
	9	0.128449732
	10	0.143542475
	11	0.160568968
PAO	1	0.090223972
	2	0.112569576
	3	0.118042698
	4	0.105842202
	5	0.086371703
	6	0.105557298
	7	0.108696996
	8	0.086811588
	9	0.082992492
	10	0.091676799

APPENDIX F

Results of soil analysis from seven sites. From each site, three 30-cm soil cores were collected in the center and perimeter of each population. Each soil core sample was divided into the top 15 cm (T) and bottom 15 cm (B). Samples from the two depths were then blended and transported on ice to be analyzed by A&L Great Lakes Laboratory in Fort Wayne, Indiana, U.S.A. Analysis measured the percentage of organic matter, pH, and nutrient levels for each site.

Population	Organic Matter	pH	P	K	MG	CA	K (%)	Mg (%)	NO_3	NH_4
NAT-T	2.3	6.8	36	54	140	700	2.8	23.6	3	8
NAT-B	0.7	6.6	34	29	120	500	2.1	28.0	1	6
NAK-T	2.1	5.7	30	39	105	400	2.4	21.0	1	8
NAK-B	0.9	5.4	18	26	65	300	2.0	16.4	1	6
LMI-T	2.4	5.9	36	36	115	450	2.1	21.3	2	11
LMI-B	1.0	6.0	47	20	95	350	1.4	20.9	1	6
LMR-T	2.3	5.7	50	43	125	500	2.3	21.5	1	8
LMR-B	1.1	5.8	46	21	75	300	1.6	18.5	1	7
PAM-T	5.9	7.4	8	64	360	2450	1.1	19.5	9	7
PAM-B	2.8	7.9	4	40	380	3200	0.5	16.4	3	6
PAO-T	4.6	6.1	9	76	380	1300	1.6	25.8	3	9
PAO-B	1.6	5.3	8	40	340	700	0.9	25.2	1	7
FUL-T	3.7	7.1	12	44	350	1150	1.3	33.2	4	16
FUL-B	2.0	7.1	9	19	295	850	0.7	36.4	1	9